MPSA 2008 Sapporo

17th Meeting of Methods in Protein Structure Analysis

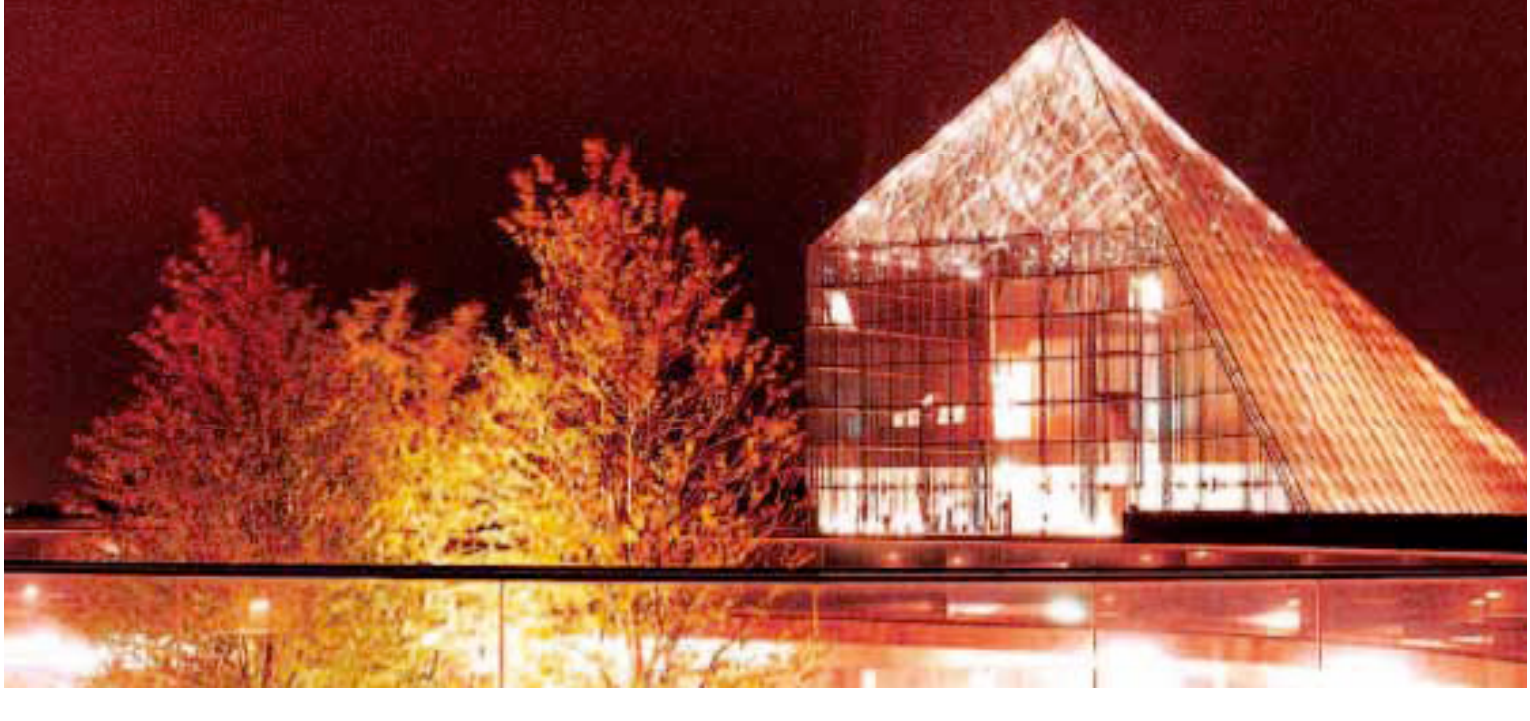
August 26–29, 2008 Sapporo, JAPAN

The International Association for Protein Structure Analysis and Proteomics

MPSA2008

Chemical and Structural Proteomics: The Solution to Biological Problems!

PROGRAM
ABSTRACT



August 26, 2008

Greetings,

On behalf of the MPSA2008 Organizing Committee, I am pleased to welcome you to the 17th Meeting of Methods in Protein Structure Analysis (MPSA2008) held in Sapporo, Japan.

MPSA meetings are biannual, international meetings held alternately in the US, Europe, and occasionally other countries. The MPSA conferences began in 1974 with a small workshop in Boston, MA, USA. Since 2000, MPSA conferences have been sponsored by the International Association for Protein Structure Analysis and Proteomics (IAPSAP). Over time they have grown and involved in scope, but the mission of IAPSAP has remained to promote the discovery and exchange of new methods and techniques for the analysis of protein structure, and to facilitate the application of methods in protein structure analysis in the pursuit of solutions to biological problems.

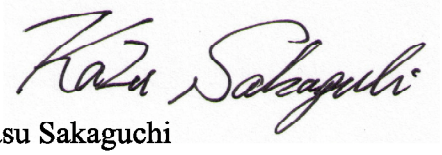
The first MPSA conference in Japan, MPSA1992, was organized by Kazutomo Imahori and Fumio Sakiyama. After 16 years, the MPSA conference has returned to Japan. MPSA2008 is designed to introduce students and researchers at all levels to the rapid advances that are occurring in the field of proteomics and the technologies for protein structure and function analysis, under the theme "Chemical and Structural Proteomics: The Solution to Biological Problems!" Participants will experience four days of presentations, workshops and discussions on key issues and vital biological problems that are being addressed through protein structure analysis and proteomics.

The logo for MPSA2008 was designed to reflect four cornerstones of the conference -
"M" represents the Protein "Molecule", "P" the "Polypeptide" of which the protein is composed, "S" is for "Structure", both primary and secondary, and "A" is for "Association", reflecting the network of interactions that result in biological function.

I strongly believe we will see at MPSA2008 the future of protein science through the cutting-edge research that will be presented and the extensive interactions among scientists from many fields that will occur at this meeting.

Sapporo is a young city and has a rich mixture of both modern culture and wonderful nature - a perfect site to host a meeting in this young but rapidly growing field. Sapporo is well known internationally for hosting the 11th Winter Olympic Games and for beer brewing. I welcome you to Sapporo, our city, and hope you will have the opportunity to enjoy its unique flavor. Let the science games begin!

For the organizing committee,



Kazuyasu Sakaguchi
Chair, MPSA2008 Organizing Committee

Congress Outline

Details

Congress
17th Meeting of Methods in Protein Structure Analysis (MPSA2008)

Theme
"Chemical and Structural Proteomics: The Solution to Biological Problems!"

Date
August 26-29, 2008

Venue
Conference Hall, Hokkaido University, Sapporo, JAPAN

Chair
Kazuyasu Sakaguchi (Professor, Hokkaido University)

Vice-chair
Hisashi Hirano (Professor, Yokohama City University)

Organizers
The International Association for Protein Structure Analysis and Proteomics (IAPSAP)
MPSA2008 Organizing Committee

Website
http://www.mpsa2008.jp/

Partners

The Japanese Biochemical Society (JBS)
The Pharmaceutical Society of Japan (PSJ)
The Chemical Society of Japan (CSJ)
The Japan Society for Analytical Chemistry (JSAC)
The Mass Spectrometry Society of Japan (MSSJ)
The Japanese Electrophoresis Society (JES)
The Japanese Society for Bioinformatics (JSBi)

Sponsor

The Federation of Pharmaceutical Manufacturers' Associations of JAPAN

Supporters

THE AKIYAMA FOUNDATION
IKETANI SCIENCE AND TECHNOLOGY FOUNDATION
The Kao Foundation for Arts and Sciences
The ITO Foundation for the Promotion of Medical Sciences

Cooperated by

Hokkaido
City of Sapporo
Sapporo international communication Plaza

MPSA Organization

MPSA2008 International Organizing Committee

Fumio Sakiyama, Chair (IAPSAP, Japan)
Carl W. Anderson (Brookhaven National Laboratory, USA)
Ettore Appella (NIH, USA)
Mitiko Go (Ochanomizu University, Japan)
Hisashi Hirano (Yokohama City University, Japan)
Kazuyasu Sakaguchi (Hokkaido Univcrsity, Japan)
Yasutsugu Shimonishi (Nagahama Institute of Bio-Science and Technology, Japan)
Koichi Suzuki (New Frontiers Research Laboratories, Toray Industries, Inc., Japan)
Naoyuki Taniguchi (Osaka University, Japan)

MPSA2008 Local Organizing Committee

Kazuyasu Sakaguchi, Chair (Hokkaido University, Japan)
Hisashi Hirano, Vice-chair (Yokohama City University, Japan)
Toshinori Endo (Hokkaido University, Japan)
Masanori Hatakeyama (Hokkaido University, Japan)
Fuyuhiko Inagaki (Hokkaido University, Japan)
Koichiro Ishimori (Hokkaido University, Japan)
Chikashi Obuse (Hokkaido University, Japan)
Isao Tanaka (Hokkaido University, Japan)
Michio Yazawa (Hokkaido University, Japan)
Hideyoshi Yokosawa (Hokkaido University, Japan)

MPSA2008 International Scientific Advisers

Ruedi H. Aebersold (Swiss Federal Institute of Technology, Switzerland)
Juan J. Calvete (C.S.I.C., Spain)
Theodora Choli-Papadopoulou (Aristotle University of Thessaloniki, Greece)
Jay W. Fox (University of Virginia Medical School, USA)
Jan Johansson (Swedish University of Agricultural Sciences, Sweden)
Hans Jörnvall (Karolinska Institutet, Sweden)
Nisse Kalkkinen (Institute of Biotechnology, Finland)
Roza M. Kamp (University of Applied Sciences, Germany)
Gabriel Padrón Palomares (Centro de Ingenieria Genetica y Biotecnologia, Cuba)
Guy Lippens (Universite des Sciences et Technologies de Lille, France)
Richard N. Perham (St John's College, University of Cambridge, UK)
Richard Simpson (Royal Melbourne Hospital, Australia)
Harald Tschesche (University of Bielefeld, Germany)
Joël Vandekerchkhove (Ghent University, Belgium)
Brigitte Wittmann-Liebold (Wita GmbH, Germany)
Satya Yadav (The Cleveland Clinic Foundation, USA)

MPSA Organization

MPSA2008 Program Advisers

Norie Araki (Kumamoto University, Japan)
Yoshifumi Fukunishi (Japan Biological Information Research Center (BIRC-AIST), Japan)
Yuji Goto (Osaka University, Japan)
Shigetsugu Hatakeyama (Hokkaido University, Japan)
Hiroshi Hirota (RIKEN (The Institute of Physical and Chemical Research), Japan)
Toshiaki Isobe (Tokyo Metropolitan University, Japan)
Takumi Kamura (Nagoya University, Japan)
Takeshi Kawabata (Nara Institute of Science and Technology, Japan)
Hiroshi Kawasaki (Yokohama City University, Japan)
Nana Kawasaki (National Institute of Health Sciences, Japan)
Yasushi Kawata (Tottori University, Japan)
Akinori Kidera (Yokohama City University, Japan)
Makoto Kimura (Kyushu University, Japan)
Masataka Kinjo (Hokkaido University, Japan)
Yuji Kobayashi (Osaka University of Pharmaceutical Sciences, Japan)
Tadashi Kondo (National Cancer Center Research Institute, Japan)
Seiki Kuramitsu (Osaka University, Japan)
Tadakazu Maeda (Kitasato University, Japan)
Kunio Miki (Kyoto University, Japan)
Koji Nagao (Okinawa Institute of Science and Technology, Japan)
Atsushi Nakagawa (Osaka University, Japan)
Haruki Nakamura (Osaka University, Japan)
Kazuyuki Nakamura (Yamaguchi University, Japan)
Toyofumi Nakanishi (Osaka Medical College, Japan)
Takashi Nakazawa (Nara Women's University, Japan)
Yoshifumi Nishimura (Yokohama City University, Japan)
Hiroyuki Noji (Osaka University, Japan)
Fumio Nomura (Chiba University, Japan)
Yoshiya Oda (Eizai Co., Ltd., Japan)
Masamichi Ohishi (Kitasato University, Japan)
Motonori Ota (Tokyo Institute of Technology, Japan)
Yasuyuki Shimohigashi (Kyushu University, Japan)
Ryo Taguchi (The University of Tokyo, Japan)
Shoji Takada (Kyoto University, Japan)
Nobuhiro Takahashi (Tokyo University of Agriculture and Technology, Japan)
Toshifumi Takao (Osaka University, Japan)
Masaru Tanokura (The University of Tokyo, Japan)
Tosifusa Toda (Tokyo Metropolitan Institute of Gerontology, Japan)
Tomitake Tsukihara (Osaka University, Japan)
Yoshinao Wada (Osaka Medical Center and Research Institute for Maternal and Child Health, Japan)
Soichi Wakatsuki (High Energy Accelerator Research Organization, Japan)
Tesshi Yamada (National Cancer Center Research Institute, Japan)
Toshio Yanagida (Osaka University, Japan)
Shigeyuki Yokoyama (The University of Tokyo, Japan)
Minoru Yoshida (RIKEN (The Institute of Physical and Chemical Research), Japan)
Katsutoshi Yoshizato (Hiroshima University, Japan)

(Alphabetical Order)

Registration

Registration Desk

The Registration Desk will be set up on the Entrance of the Conference Hall.

Opening Hours

Date	Registration
Aug. 26	15:00-17:00
Aug. 27	8:15-17:00
Aug. 28	8:15-17:00
Aug. 29	8:15-16:00
Aug. 30	10:00-Tour

Registration Fees

(yen / person)

Category	Onsite
Regular	60,000
Post-doctor	30,000
Student	20,000
Accompanying person (Welcome Reception)	3,000
Japanese Kimono Wearing (Aug. 27)	3,000
Banquet (Aug. 28)	8,000
Tour (Aug. 30)	7,000

Credit Cards and Exchange

Only Japanese currency and credit cards (VISA, MasterCard, American Express) will be accepted.
Major foreign currencies can be exchanged at the Bank and Hotel.

Congress Kit

A Congress Kit will be handed to participants at the Registration Desk.

Contents:
- Program / Abstract
- Nameplate
- Banquet ticket(s) (Only for those who paid in advance)
- Tour ticket(s) (Only for those who paid in advance)
- Receipt
- Invitation Letter
- Certificate
- Tourist Guidebook
- Participants List

Nameplate

Participants must wear their nameplates at all times at the Congress site.

Banquet Ticket

Tickets for the banquet (Aug. 28) can be purchased at the Registration Desk (if vacant seats are available).
The banquet admission fee is 8,000 yen. Cancellation and refund will not be accepted.

Accommodations/Tour Desk

The Accommodations and Tour Desk will be set up next to the Registration Desk, where JTB staff will be stationed to welcome you.
Accommodations/Tour Desk: 10:00-17:00
Tour reservation: until the morning of Aug. 29

Cloakroom

The cloakroom will be opened on the Conference Hall 1F. Please be sure to retrieve any items you have checked at the cloakroom on the same day.

Official Language

English is the official language.

To Presenters and Chairpersons

About oral presentations

To Chairpersons

The chairpersons should report their arrival at the site to the reception desk for chairpersons 30 minutes prior to the beginning of each session. At the site, please use the seats prepared for the co-chairpersons in the right-front side of the venue 15 minutes before the start time.

General guideline for presentation time

Keynote Lecture:
60 min presentation
Pleanry Lecture: 40 min
(35 min presentation + 5 min discussion)
Lecture Session: 35 min
(30 min presentation + 5 min discussion)
Short Talks: 17 min
(14 min presentation + 3 min discussion)
Young Scientist Talks: 12 min
(9 min presentation + 3 min discussion)

Guideline for Speakers

Please report your arrival at the site to the reception desk 60 minutes prior to the beginning of each session.
Please present yourself at the session room at least 15 minutes before your session to meet the chairpersons and to prepare for your presentation.
Chairpersons will come to the room 15 minutes before the start time.

Prcparing Your Prcscntation;

Official Language for the conference is English. Please prepare your presentation in English. No dedicated template is prepared. Please use your own template.

Equipments for Presentation;

ALCD projector (XGA:1024 × 768 pixels), a projector screen, microphones and a laser pointer will be provided in all the session rooms.
Please use your own PC to avoid troubles in reading and displaying data.
In case you cannot bring your PC, you may use a seretarist's PC prepared in the session room with your USB memory. FD, CD, MO or ZIP is not available. MS-Windows XP and MS-PowerPoint 2003 are installed in the secretalist's PCs. We also recommend the Macintosh and Windows Vista users to use their own PCs.

Prior to the Session;

Every speaker has to make a biography sheet for education and professional career, and hand over it to the chairperson.
In advance of the session start, please connect your PC to the selector box and confirm that the data are exactly displayed.

About poster presentations

Poster Discussion

Presenters for poster sessions should be present in front of their poster boards according to the schedule specified below.

During the presentation time, Program Committee Members will visit the poster presentation sites to evaluate your posters to determine which posters are to be nominated as the best poster awards.

Poster Presentation Time

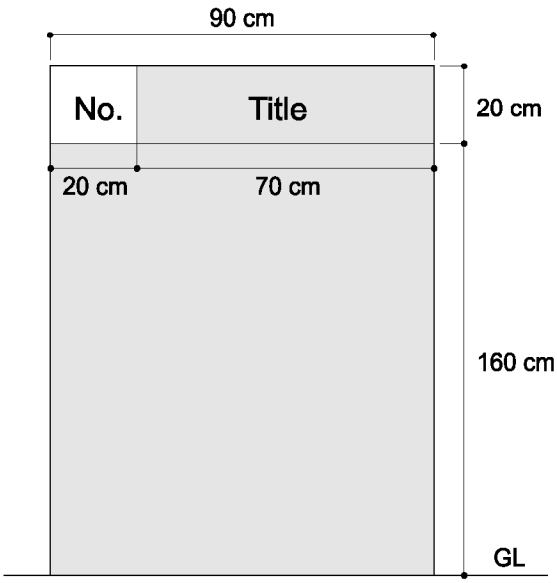
Date	Time	Topics number
Aug. 27	17:25-18:55	Odd number only
Aug. 28	17:20-18:50	Even number only

Mounting/Removal Time of Posters

1) Mounting time: 15:00 - 18:00 on Aug. 26 or 9:00 on Aug. 27
2) Removal time: 12:00 on Aug. 29
3) Please note that posters have not been removed by the end of the removal time.

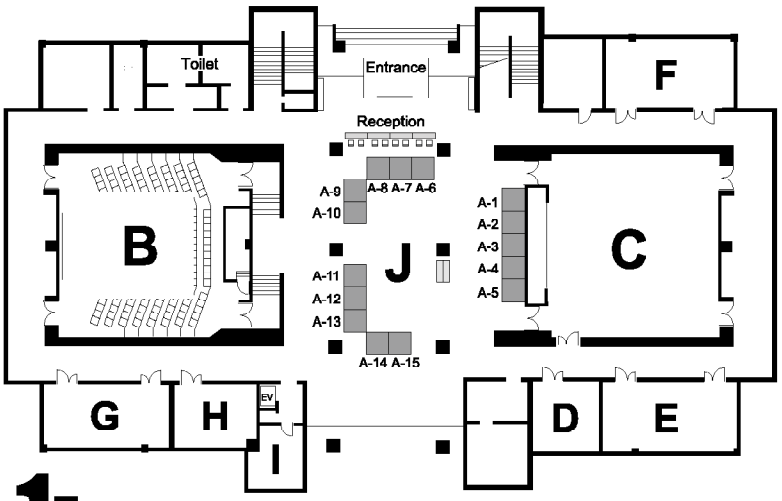
Poster Panel

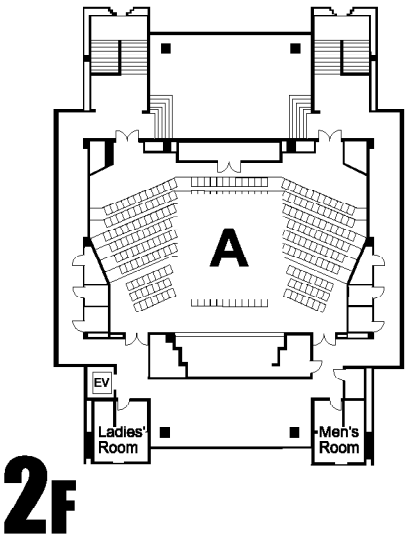
On Poster Panels (180 cm high × 90 cm wide) the title, author(s) and institution(s) (20 cm high × 70 cm wide) must be displayed as shown in the figure.
Title paper should be prepared by presenter.
Poster number is put by organizer.



Conference Site

Hokkaido University Conference Hall





A: Conference Hall A
B: Conference Hall B
C: Poster Presentation
D: Secretariate Room
E: Secretariate Room
F: Cloak Room
G: PC Check Room
Inter-net Service
H: Meeting Room
I: Meeting Room

J: Exhibition
A-1. Invitrogen Japan K.K
A-2. Matrix Science K.K.
A-3. Nihon Waters K.K.
A-4. Applied Biosystems Japan Ltd.
A-5. GE Healthcare Bio-Sciences KK
A-6. Merck Ltd., Japan
A-7. ThermoFisher Scientific
A-8. Nihon Millipore K.K.
A-9. Nippon Dionex K.K.
A-10. Thermo Fisher Scientific
A-11. Agilent TechnologiesJapan,Ltd
A-12. TOSOH CORPORATION
A-13. Wako Pure Chemical Industries
A-14. SHIMADZU CORPORATION
A-15. Hitachi High-Technologies Corporeation

Social Events and Other

Japanese Tea Ceremony & Kimono Wearing

Date: August 27 (Wed.)

- Japanese Tea Ceremony
  Volunteer instructors in the Japanese Tea Ceremony will help visitors to the Sapporo Convention Bureau experience the authentic Japanese tea ceremony. This is an excellent informal opportunity to learn about the utensils used for making tea as well as the etiquette of serving it.
- Kimono Wearing (Fee: 3,000 yen)
  Volunteer kimono instructors will help visitors to the Sapporo Convention Bureau experience how to put on a kimono. Participants can also have their photos taken wearing the kimonos.

Social Program

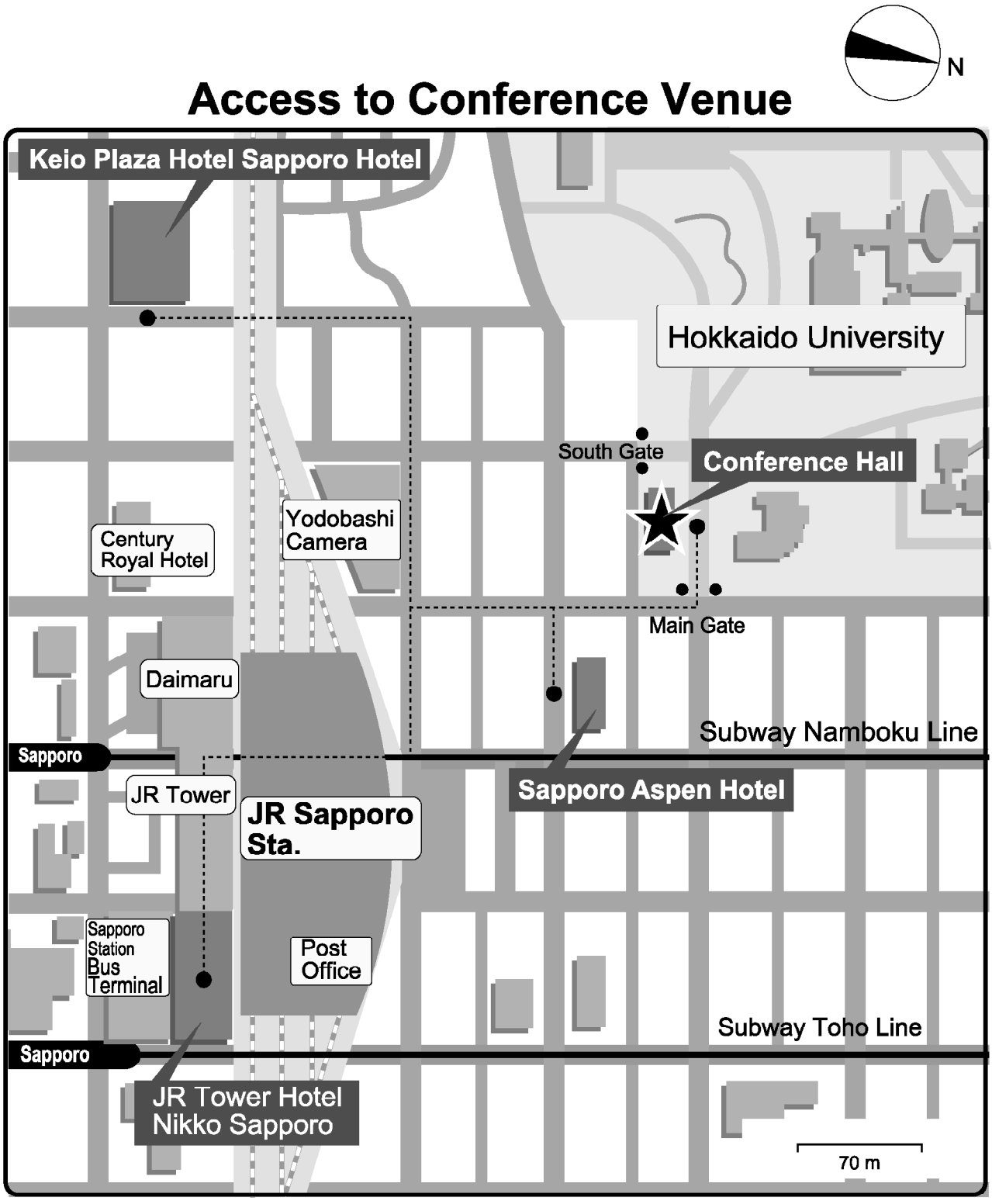
- Welcome Reception (free)
  Aug. 26, 2008 18:35-20:00
  Aspen Hotel Banquet Hall
  Tel: 011-700-2111
- Banquet (8,000 yen)
  Aug. 28, 2008 19:00-21:00
  JR Tower Hotel Nikko Sapporo
  Tel: 011-251-2222
- Tour (7,000 yen)
  Aug. 30, 2008 10:00-16:00
  Sapporo Neighborhood

Access to New Chitose Airport

- Train
  Get on JR train "Rapid Airport" for New Chitose Airport about 36 min.
  Fare: 1,040 yen.
- Bus
  Airport Bus from major hotel to New Chitose Airport about 90 min.
  Fare: adult 1,000 yen child 500 yen

Map

Access to Conference Venue



- Aug. 26, 2008 18:35-20:00
  Welcome Reception: Aspen Hotel Banquet Hall

- Aug. 28, 2008 19:00-21:00
  Banquet: JR Tower Hotel Nikko Sapporo 36F

Acknowledgements

The Federation of Pharmaceutical Manufacturers' Associations of JAPAN
日本製薬団体連合会

Thermo SCIENTIFIC
Applied Biosystems
Waters THE SCIENCE OF WHAT'S POSSIBLE.™
Wako
Agilent Technologies
日立ハイテク HITACHI
GE Healthcare
MERCK
SHIMADZU
DIONEX
MILLIPORE
invitrogen™
MATRIX SCIENCE
TOSOH
AMR AMR INCORPORATED
アズワン株式会社
anet
MSイムノサイエンス株式会社
SAPPORO
千歳鶴

Program

	Aug. 26 (Tue)	Aug. 27 (Wed)	Aug. 28 (Thu)	Aug. 29 (Fri)
8:00				
8:30		8:30 Session 1: Chemical Proteomics L-01, L-02, L-03	8:20 Short Talks and Young Scientist Talks (II) Y-04, Y-05, Y-06, Y-07, Y-08, Y-09 S-01, S-02	8:20 Short Talks and Young Scientist Talks (III) Y-10, Y-11 S-03, S-04, S-05
9:00				
9:30				9:40 *Coffee break*
10:00		10:15 *Coffee break*	10:15 *Coffee break*	10:05 Session 6: Technology for Protein Structure & Function L-16, L-17, L-18, L-19
10:30		10:40 Session 2: Structural Proteomics L-04, L-05, L-06	10:40 Session 4: Protein Interaction & Bioinformatics L-11, L-12, L-13	
11:00				
11:30				
12:00				
12:30	12:30 Pre-conference Workshop (in Japanese only)	12:30 Luncheon Seminar 1 Luncheon Seminar 2	12:30 Luncheon Seminar 3 Luncheon Seminar 4	12:30 Luncheon Seminar 5 Luncheon Seminar 6
13:00				
13:30				
14:00		13:55 Short Talks and Young Scientist Talks (I) Y-01, Y-02, Y-03	13:55 Session 5: Post-translational Modification L-14, L-15	13:55 Short Talks and Young Scientist Talks (IV) S-06, S-07
14:30		14:40 Session 3: Glyco-proteomics L-07, L-08	15:05 Young Investigator Award Lecture	14:30 Session 7: Clinical Proteomics & Novel Technology for Biomarkers L-20, L-21
15:00			15:35 *Coffee break*	Closing Plenary Lecture PL-1
15:30		15:50 *Coffee break*	16:00 Edman Award Lectures	Closing Remarks
16:00		16:15 continue Session 3: Glyco-proteomics L-09, L-10		
16:30				
17:00				
17:30	17:30 Opening Remarks Keynote Lecture KL-1	17:25 Poster Session (A) (Odd number)	17:20 Poster Session (B) (Even number)	
18:00				
18:30	18:35 Welcome Reception (Aspen Hotel)			
19:00			19:00 Banquet (JR Tower Hotel Nikko)	
19:30				
20:00				
20:30				

Program

Lecture --- Conference Hall A

Poster --- Conference Room C

Aug. 26 (Tue)

12:30-16:30
Pre-conference Workshop (in Japanese only)

17:30-18:35
Opening Remarks
Keynote Lecture
(Chairperson: Ettore Appella)
KL-01 Degradomics: the proteolysis of cell death
James A. Wells (UCSF, USA)

18:35-
Welcome Reception (Aspen Hotel)

Aug. 27 (Wed)

8:30-10:15
Session 1: Chemical Proteomics
(Chairpersons: Carl W. Anderson, Yasuyuki Shimohigashi)
L-01 Molecular networks, systems medicine and the mechanism of action of drugs
Giulio Superti-Furga (Austrian Academy of Sciences, Austria)
L-02 Chemical genomics of bioactive natural products based on the fission yeast ORFeom
Minoru Yoshida (RIKEN, Japan)
L-03 Chemical proteomics-based strategies in drug discovery
Gerard Drewes (Cellzome AG, Germany)

10:15-10:40
Coffee break

10:40-12:25
Session 2: Structural Proteomics
(Chairpersons: Jan Johansson, Yasushi Kawata)
L-04 Diversity & specificity in protein ubiquitination: the BRCA1/BARD1 paradigm
Rachel E. Klevit (University of Washington, USA)
L-05 Prenyltransferases as targets for the discovery of new antibiotics
Andrew H.-J. Wang (Academia Sinica, Taiwan)
L-06 The role of structural disorder in protein-protein interactions
Péter Tompa (Hungarian Academy of Sciences, Hungary)

12:30-13:30
Luncheon Seminar
LS-1 LC/MS/MS tools for complex glycan and glycopeptide characterization in bio-therapeutics
Sponsored by Applied Biosystems (Conference Hall A)
LS-2 Brand New Biomarkers for Prostate Cancer and Diabetes
Sponsored by Wako Pure Chemical Industries, Ltd. (Conference Hall B)

13:55-14:35
Short talks and Young Scientist Talks (I)
(Chairperson: Theodora Choli-Papadopoulou)
Y-01 Structural flexibility of human nuclear receptor ERRγ to adopt endocrine disruptor bisphenol A and its derivatives
Ayami Matsushima (Kyushu University, Japan)
Y-02 Three-dimensional structure of gastric H/K-ATPase at 6.5 Å resolution determined by electron crystallography of two-dimensional crystals
Kazuhiro Abe (Kyoto University, Japan)
Y-03 Xyloglucan recognition mechanism suggested from the crystal structure of Cel44A
Yu Kitago (Hokkaido University, Japan)

14:40-15:50
Session 3: Glyco-proteomics
(Chairpersons: Naoyuki Taniguchi, Brigitte Wittmann-Liebold)
L-07 GLYCOMICS: Same tools, same questions, different answers
Nicolle H. Packer (Macquarie University, Australia)
L-08 Modification specific proteomics applied to analysis of protein glycosylation
Peter Roepstorff (University of Southern Denmark, Denmark)

15:50-16:15
Coffee break

16:15-17:25
continue Session 3: Glyco-proteomics

L-09 Discovery of glyco-biomarkers and their biological function using novel glycomics technologies
Hisashi Narimatsu (AIST, Japan)

L-10 Strategies for analysis of glycoprotein glycosylation
Rudolf Geyer (Justus-Liebig-University Giessen, Germany)

17:25-18:55
Poster Session (A) (Odd number)

Aug. 28 (Thu)

8:20-10:15
Short Talks and Young Scientist Talks (II)
(Chairpersons: Jung Sup Lee, Masayuki Takahashi)

Y-04 New approaches for developing therapeutic drugs against neutrophil activation by HPNAP from Helicobacter pylori
Antonios Asiminas (Aristotle University, Greece)

Y-05 Structural and functional analysis of a novel, highly conserved, glomerulus specific protein shisa3
Juha R.M. Ojala (Karolinska Institutet, Sweden)

Y-06 Alteration of *Streptomyces clavuligerus* deacetoxycephalosporin C synthase substrate binding pocket to accommodate penicillins with aromatic side chains
Kian Sim Goo (National University of Singapore, Singapore)

Y-07 Toll-like receptors 2 and 4 can be activated by vEP protease to induce an inflammatory response
Jung Eun Park (Chosun University, Republic of Korea)

Y-08 An alternative splicing variant of the PPM1 protein phosphatase PPM1D
Yoshiro Chuman (Hokkaido University, Japan)

S-01 Secondary structure mimics of protein-protein interactions
Siddhartha Roy (Indian Institute of Chemical Biology, India)

Y-09 Landscaping enzymatic reactions of oxidoreductases by a self-organizing neural network
Keisuke Ueno (Hokkaido University, Japan)

S-02 Gene annotation in *Toxoplasma gondii*
Ruth Hogue Angeletti (Albert Einstein College of Medicine, USA)

10:15-10:40
Coffee break

10:40-12:25
Session 4: Protein Interaction & Bioinformatics
(Chairpersons: Toshiaki Isobe, Roza M. Kamp)

L-11 Global mapping of genetic and chemical-genetic networks in yeast
Charles Boone (University of Toronto, Canada)

L-12 Proteomic characterization of human pre-ribosome particles
Nobuhiro Takahashi (Tokyo University of Agriculture and Technology, Japan)

L-13 Protein interaction specificity: using 3D structure to unveil compensatory effects in protein interaction networks
Patrick Aloy (IRB Barcelona, Spain)

12:30-13:30
Luncheon Seminar

LS-3 New developments in ETD on LTQ Orbitrap XL and its applications
Sponsored by Thermo Fisher Scientific Inc. (Conference Hall A)

LS-4 High Definition Mass Spectrometry: A New way to Visualise Peptides, Proteins and Protein Complexes in Top-Down/ Bottom-Up Proteomics.
Sponsored by Nihon Waters K.K. (Conference Hall B)

13:55-15:05
Session 5: Post-translational Modification
(Chairpersons: Yasushi Ishihama, Masamichi Ohishi)

L-14 Innovative mass spectrometry technology for the study of cell signaling
Donald F. Hunt (University of Virginia, USA)

L-15 Novel MS-based proteomics technologies for dissection of post-translational modification networks
Yingming Zhao (UT Southwestern Medical Center, USA)

15:05-15:35
Young Investigator Award Lecture
(Chairperson: Ettore Appella)
Andreas Ladurner (EMBL, Germany)

15:35-16:00
Coffee break

16:00-17:20
Edman Award Lectures
(Chairperson: Fumio Sakiyama; Award Committee Chair: Hans Jörnvall)
Richard Perham (University of Cambridge, UK)
Ettore Appella (National Institutes of Health, USA)

17:20-18:50
Poster Session (B) (Even number)

19:00-21:00
Banquet at JR Tower

Aug. 29 (Fri)

8:20-9:40
Short talks and Young scientist talks (III)
(Chairpersons: Takashi Nakazawa, Satya Yadav)
Y-10 Single molecule dynamics studied with atomic force microscopy: The effect of temperature on the energy landscape of mechanical unfolding of proteins
Yukinori Taniguchi (Japan Advanced Institute of Science and Technology, Japan)
Y-11 Selective extraction and enrichment of multi-phosphorylated peptides using polyarginine-coated diamond nanoparticles
Chih-Che Wu (National Chi Nan University, Taiwan)
S-03 Liquid chromatography - mass spectrometry using ion trap based electron capture dissociation
Takashi Baba (University of North Carolina, USA)
S-04 Defining a new route to absolute protein quantitation
Mark McDowall (Waters Corporation, England)
S-05 Targeted LC/MS/MS techniques characterize recombinant therapeutics, including heterogeneity, and low-frequency post translational modifications
Matthew M. Champion (Applied Biosystems, USA)

9:40-10:05
Coffee break

10:05-12:25
Session 6: Technology for Protein Structure & Function
(Chairpersons: Richard N. Perham, Kazuki Saito)
L-16 Linking protein arrays to signalling pathways and diseases
Jan van Oostrum (Zeptosens, Switzerland)
L-17 Mechanical modulation of ATP-binding and hydrolysis by single F1-ATPase molecule
Hiroyuki Noji (Osaka University, Japan)
L-18 Genetic code reprogramming and RaPID system
Hiroaki Suga (University of Tokyo, Japan)
L-19 Proteins in action: Monitored by tr(time-resolved) FTIR spectroscopy
Klaus Gerwert (Ruhr University Bochum, Germany)

12:30-13:30
Luncheon Seminar
LS-5 Electron capture dissociation in RF trap based ECD - TOF mass spectrometer
Sponsored by Agilent Technologies (Conference Hall A)
LS-6 HPLC-Chip combined with triple quadrupole MS for high sensitivity peptide quantitation
Sponsored by Hitachi High-Technologies Corporation (Conference Hall B)

13:55-14:30
Short talks and Young Scientist Talks (IV)
(Chairperson: Ruth H. Angeletti)
S-06 RNA-binding CCCH-type zinc-finger proteins are essential for oocyte maturation in *C. elegans*
Hiroyuki Kawahara (Tokyo Metropolitan University, Japan)
S-07 Analysis of specific proteins related to chemotherapy sensitivities in gliomas by assembled proteomic strategies
Norie Araki (Kumamoto University, Japan)

14:30-16:30
Session 7: Clinical Proteomics & Novel Technology for Biomarkers
(Chairpersons: Jay W. Fox, Masanori Hatakeyama)
L-20 Proteomics identification of proteins involved in colorectal cancer metastasis
Maxey Ching Ming Chung (National University of Singapore, Singapore)
L-21 Cancer proteomics for personalized medicine
Tadashi Kondo (National Cancer Center, Japan)

Closing Plenary Lecture

(Chairperson: Yasutsugu Shimonishi)

PL-1 Proteomic strategies for biomarker discovery and validation
Stephen R. Pennington (University College Dublin, Ireland)

Closing Remarks

Aug. 30 (Sat)

Excursion

POSTER PRESENTATIONS

P-001

Analysis of lactate dehydrogenases (LDHs) from hagfishes in high pressure conditions by high-pressure electrophoresis and high-pressure photometry

Yoshikazu Nishiguchi[1], Fumiyoshi Abe[2], Akira Uchida[3], Tetsuya Miwa[2], Chiaki Kato[2], Takako Sato[2], Souichirou Kubota[3], Yasui Kinya[4], Noriko Oshima[3], Hiroyuki Satoh[3], Muneo Morishita[1], Michiko Goromaru-Shinkai[1], Nobue Ito[3], Mitsumasa Okada[3]
[1]Faculty of Pharmaceutical Sciences, Toho University, Japan, [2]Extremobiosphere Research Center, JAMSTEC, Japan, [3]Faculty of Science, Toho University, Japan, [4]Department of Biological Science, Hiroshima University, Japan

P-002

Rapid, comprehensive and high-resolution intact protein separation for proteomics

Angelika Koepf [1], Matsamitu Okawara [2], Karl Burgess [3], Ken Cook [4], Andrew Pitt [3]
[1] Dionex Corp., Asia Pacific, Bangkok, Thailand, [2] Nippon Dionex K.K., Tokyo, Japan, [3] University of Glasgow, Glasgow,, [4] Dionex Corp., Camberley, UK

P-003

An efficient screening method for protein-binding peptides using capillary electrophoresis-mass spectroscopy (CE-MS)

Kazuki Saito[1], Mamiko Nakato[2], Takaaki Mizuguchi[2], Hiromasa Uchimura[1], Shigeyuki Yokoyama[3], Hiroshi Hirota[3], Yoshiaki Kiso[2]
[1]Laboratory of Proteomic Sciences, 21st Century COE Program, Kyoto Pharmaceutical University, Japan, [2]Department of Medicinal Chemistry, Center for Frontier Research in Medicinal Science and 21st Century COE Program, Kyoto Pharmaceutical University, [3]Protein Research Group, RIKEN Genomic Sciences Center, Japan

P-004

Using a mapping function into a higher-dimensional space for filtering criteria against SEQUEST database search results

Kouki Yonezawa [1], Katsuhiko Mineta [1], Toshinori Endo [1], Lixy Yamada [2], Hisaaki Taniguchi [2]
[1] Graduate School of Information Science and Technology, Hokkaido University, [2] The Institute for Enzyme Research, The University of Tokushima, Japan

P-005

Multiple MS fragmentation as a versatile tool for discrimination of glycan isomers: Application to the neural glycoprotein CD24

Christina Bleckmann[1], **Hildegard Geyer**[1], Ralf Kleene[2], Melitta Schachner[2], Vernon Reinhold[3], Rudolf Geyer[1]
[1]Institute of Biochemistry, Faculty of Medicine, Justus-Liebig-University Giessen, [2]Center of Molecular Neurobiology, University of Hamburg, [3]Division of Molecular, Cellular, & Biomedical Sciences, University of Hampshire, Germany

P-006

Protein sequencing analysis using MALDI-TOF-MS and chemical modifications of tryptic peptides

Tomohiro Araki[1], Koichi Sakakibara[1], Atsushi Matsuoka[1], Norie Araki[2]
[1]School of Agriculture, Tokai University, [2]Graduate School of Medical Sciences, Kumamoto University, Japan

P-007 (Y-11)

Selective extraction and enrichment of multi-phosphorylated peptides using polyarginine-coated diamond nanoparticles

Chih-Che Wu[1], Chia-Kai Chang[1], Yi-Sheng Wang[2], Huan-Cheng Chang[3]
[1]Department of Applied Chemistry, National Chi Nan University, [2]Genomics Research Center, Academia Sinica, [3]Institute of Atomic and Molecular Sciences, Academia Sinica, Taiwan

P-008

Identification and characterization of novel human ORC binding proteins

Masahiro Yamaguchi[1], Satosi Ohta[3], Takasi Seki[1], Koji Nagao[2], Chikasi Obuse[1]
[1]Faculty of Advanced Life Science Hokkaido University, [2]G0 Cell Unit, Initial Research Project, Okinawa Institute of Science and Technology, [3]Structural Biochemistry, Jichi Medical University, Japan

P-009

A mechanism for heme-mediated oxidative modification of iron response regulator

Chihiro Kitatsuji[1], Atsushi Nakamura[2], Masaki Kurogochi[3], Shin-Ichiro Nishimura[3], Mark R. O'Brian[4], Koichiro Ishimori[2]
[1]Graduate School of Engineering, Kyoto University, [2]Graduate School of Science, Hokkaido University, [3]Graduate School of Life Science, Hokkaido University, Japan, [4]Department of Biochemistry, State University of New York at Buffalo, USA

P-010

Facile MALDI-MS analysis of neutral glycans in NaOH-doped matrices: Microwave-assisted deglycosylation and one-step purification with diamond nanoparticles

Yan-Kai Tzeng, Cheng-Chun Chang, Chau-Chung Han, Huan-Cheng Chang
Institute of Atomic and Molecular Sciences Academia Sinica, Taiwan

P-011

Effective recovery of bioactive protein from denaturated form using microfluidic channel

Hiroshi Yamaguchi, Masaya Miyazaki, Hideaki Maeda
Nanotechnology Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Japan

P-012

Protease-immobilized microreactor for rapid protein digestion

Masaya Miyazaki, Takeshi Honda, Hiroshi Yamaguchi, Hideaki Maeda
Nanotechnology Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Japan

P-013

Conformation analysis of prion protein using fluorescence immunoconformational correlation spectroscopy (FiCS)

Hiroshi Sakata[1], Motohiro Horiuchi[2], Masataka Kinjo[1]
[1]Division of Cellular Life Science, Faculty of Advanced Life Science, Hokkaido University, [2]Laboratory of Prion Diseases, Graduate School of Veterinary Medicine, Hokkaido University, Japan

P-014

A novel method for the detection and analysis of protein pockets and cavities using a rolling probe sphere

Jian Yu, **Min Yao**, Isao Tanaka
Graduate School of Life Science, Hokkaido University, Japan

P-015

Sensitive and unequivocal determination of pKa values of individual histidine residues in proteins using mass spectrometry

Masaru Miyagi[1], Takashi Nakazawa[2]
[1]Center for Proteomics, Case Western Reserve University, USA, [2]Department of Chemistry, Nara Women's University, Japan

P-016

Multicolor detection of posttranslational modifications of proteins on a single 2-DE pattern by quantum dot technology

Masamichi Oh-Ishi, Yoshio Kodera, Tadakazu Maeda
Department of Physics, Kitasato University School of Science, Japan

P-017

Isolation of C-terminal peptides of proteins by exhaustive amidation followed by proteolytic digestion for sequencing with mass spectrometry

Mariko Nakagawa[1], Minoru Yamaguchi[2], Hiroki Kuyama[3], Chihiro Nakajima[3], Eiji Ando[2], Susumu Tsunasawa[3], Osamu Nishimura[3], Takashi Nakazawa[1]
[1]Department of Chemistry, Nara Women's University, [2]Life Science Laboratory, Shimadzu Corporation, [3]Institute for Protein Research, Osaka University, Japan

P-018

One-pot incorporation of various functional groups into polypeptide chains by RNA catalysts

Masaki Ohuchi[1], Hiroshi Murakimi[2], Hiroaki Suga[3]
[1]Department of Advanced Iterdisciplinary Studies, Graduate School of Engineering, The University of Tokyo, [2]Reserch Center for Advanced Science and Technology, The University of Tokyo, [3]Department of Chemistry and Biotechnology, Graduate School of Engineering, The University of Tokyo, Japan

P-019

Post-genome analysis of ailkworm (Bombyx mori) and the construction of proteome database

Hideyuki Kajiwara[1], Atsue Imamaki[2], Masatoshi Nakamura[1], Kazuei Mita[1], Chikatada Satoh[3], Yuji Shimizu[3], Michihiko Shimomura[3], Masumi Ishizaka[4]
[1]National Institute of Agrobiological Sciences, [2]Environmental Research Center, [3]Mitsubishi Space Software, [4]National Institute for Agro-Environmental Sciences, Japan

P-020

Label-free quantitative proteomics analysis of kidney glomeruli in ADR-induced proteinuric mice

Masatoshi Nukui, Gunvor Alvelius and Karl Tryggvason
Division of Matrix Biology, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Sweden

P-021

Protein identification and quantification using a proteome database of liquid chromatography-mass spectrometric data

Hiroshi Kawasaki, Noriaki Arakawa, Hisashi Hirano
Supramolecular Biology, International Graduate School of Arts and Sciences, Yokohama City University, Japan

P-022

An approach to the quantitative analysis of free thiol levels in human CSF proteins by the method of 2-D gel fluoroimaging with using cyanine dyes and fluorescent gel stain

Tosifusa Toda[1], Megumi Nakamura[1], Hiraku Morisawa[1], Machiko Iwamoto[2], Junpei Ohkata[3], Hajime Sato[3], Hiroshi Tanaka[3], Kazutomi Kanemaru[4]
[1]Research Team for Molecular Biomarkers, Tokyo Metropolitan Institute of Gerontology,[2]Research Team for Functional Genomics, Tokyo Metropolitan Institute of Gerontology, [3]Bio-Rad Laboratories, [4] Tokyo Metropolitan Geriatric Hospital, Japan

P-023

Comprehensive analysis of functional proteins related to the neuronal differentiation in PC12 cells induced by NGF stimulation using iTRAQ method and bioinformatics with GO analysis

Daiki Kobayashi[1], Takashi Morikawa[1], jiro Kumagai[2], Anthony Wilson[1], Masayo Wilson[1], Megumi Nagayama[1], Takeru Nanbu[3], Norie Araki[1]

[1]Department of Tumor Genetics and Biology, Graduate School of Medicine, Kumamoto University, [2]Core Laboratory for Medical Research and Education, Medical School, Kumamoto University, [3]Department of Pharmacy, Kumamoto University Hospital, Japan

P-024

GH transgenic salmon proteomics

Youichi Kurata[1], Tsukasa Mori[2], Yuko Yamanaka[1], Hisashi Hirano[1]

[1]International Graduate School of Arts and Sciences, Yokohama City University, [2]College of Bioresource Sciences ,Nihon University, Japan

P-025

Differential expression profile of protein tyrosine kinases in the mouse crypt cells of the oxazolone-induced ulcerative colitis model

Shingo Semba, Yanju Ma, Tsuyoshi Katoh, Takanobu Taniguchi

Department of Biochemistry, Asahikawa Medical College, Japan

P-026

EurocarbDB: A database and software platform for glycoinformatics

Kai Maas[1], **Hildegard Geyer**[1], Rudolf Geyer[1], Alessio Ceroni[2], Stuart Haslam[2], Rene Ranzinger[3], Claus-Wilhelm von der Lieth[3], Matthew Campbell[4], Pauline Rudd[4], Thomas Luetteke[5], Bas Leeflang[5], Magnus Lundborg[6], Goran Widmalm[6], Matt Harrison[7], Kim Henrick[7]

[1]Institute of Biochemistry, Faculty of Medicine, Justus-Liebig-University of Giessen, Germany, [2]Imperial College of Science, Technology and Medicine, London, UK, [3]German Cancer Research Center, Heidelberg, Germany, [4]National Institute for Bioprocessing Research and Training, University College Dublin, Ireland, [5]Bijvoet Center, Utrecht University, Nederland, [6]University of Stockholm, Sweden

P-027

Proteomics-based protein profiles of unfertilized egg, tadpole larva and neural complex in *Ciona intestinalis* for an integrated protein database CIPRO (*Ciona intestinalis* Protein database)

Ayako Nakajima, Mia Nakachi, Mamoru Nomura, Kazuo Inaba

Shimoda Marine Research Center, University of Tsukuba, Japan

P-028

CIPRO: an integrated protein database of the ascidian Ciona intestinalis

Toshinori Endo[1], Keisuke Ueno[1], Koki Yonezawa[1], Katsuhiko Mineta[1], Koji Hotta[2], Lixy Yamada[3], Hisaaki Taniguchi[3], Michio Ogasawara[4], Yutaka Satou[5], Mamoru Nomura[6], Ayako Nakajima[6], Mia Nakachi[6], Yasunori Sasakura[6], Kazuo Inaba[6]

[1]Information Science and Technology, Hokkaido University, [2]Biosciences and Informatics, Keio University, [3]Institute of Enzyme Research, The University of Tokushima, [4]Faculty of Science, Chiba University, [5]Graduate School of Sceince, Kyoto University, [6]Shimoda Marine Research Center, Tsukuba University, Japan

P-029 (S-02)

Gene annotation in *Toxoplasma gondii*

Ruth Hogue Angeletti, Joseph Dybas, Carlos Madrid, Dmitrij Rykunov, Edward Nieves, Fa-Yun Che, Hui Xiao, Kami Kim, Louis Weiss, Andras Fiser

Biodefense Proteomics Research Center, Albert Einstein College of Medicine, USA

P-030

Single molecular tracking of cholera toxin subunit B on GM1-incorporated in self-spreading lipid bilayer

Toshinori Motegi, Baku Takimoto, Hideki Nabika, Kei Murakoshi

Department of Chemistry, Faculty of Science, Hokkaido University, Japan

P-031

Dynamic adsorption and insertion behavior of MSI-78 on a self-spreading lipid bilayer

Hideki Nabika, Masahiro Oowada, Baku Takimoto, Takao Nomura, Yoshiro Chuman, Kazuyasu Sakaguchi, Kei Murakoshi

Division of Chemistry, Graduate School of Science, Hokkaido University, Japan

P-032 (Y-10)

Single molecule dynamics studied with atomic force microscopy: The effect of temperature on the energy landscape of mechanical unfolding of proteins

Yukinori Taniguchi[1], Masaru Kawakami[1,2]

[1]School of Materials Science, Japan Advanced Institute of Science and Technology (JAIST), [2]PRESTO of Japan Science and Technology Corporation (JST), Japan

P-033

A viral mechanism for dysregulation of post-translational modification in Kaposi's sarcoma-associated herpesvirus latency

Masahiro Fujimuro[1,2], Hideyoshi Yokosawa[1,3]

[1]Biochem. Grad. School of Pharm., Hokkaido University, [2]Mol. Cell Biol., Interdisciplinary Grad. School of Medicine and Engineering, Univ. of Yamanashi, [3]Research Resources Center, Brain Science Institute, RIKEN, Japan

P-034

Large-scale protein phosphorylation profiling by high performance phosphoproteomics based on hydroxy acid-modified metal oxide chromatography (HAMMOC)

Yasushi Ishihama, Naoyuki Sugiyama, Yutaka Kyono, Koshi Imami, Takeshi Masuda, Masaru Tomita

Institute for Advanced Biosciences, Keio University, Japan

P-035

Enrichment and analysis of phosphorylated proteins in duchenne dystrophy cells

Roza M. Kamp, Jie Guo-Thuermann, Kunigunde Stephani-Kosin

Department of Biotechnology, Faculty of Life Sciences, University of Applied Sciences Berlin, Germany

P-036

N^{α}-terminal acetylation of ribosomal proteins of *Saccharomyces cerevisiae* and its function

Masahiro Kamita[1], Roza Maria Kamp[2], Hisashi Hirano[1]

[1]International Graduate School of Arts and Sciences, Yokohama City University, Japan, [2]Life Sciences and Technology, University of Applied Sciences, Germany

P-037

Posttranslational modifications of the prometastatic protein S100A4

Mads H. Haugen[1], Kjersti Flatmark[1], Svein-Ole Mikalsen[2], Gunhild M. Malandsmo[1]

[1]Department of Tumor Biology and [2]Department of Cancer Prevention, Institute for Cancer Research, Norwegian Radium Hospital, Rikshospitalet University Hospital, Oslo, Norway

P-038

Post-translational modification of Chk2 by SUMO, a ubiquitin-like protein

Hidetaka Hosono, Hideyoshi Yokosawa

Faculty of Pharmaceutical Sciences, Hokkaido University, Japan

P-039 (Y-02)

Three-dimensional structure of gastric H/K-ATPase at 6.5 Å resolution determined by electron crystallography of two-dimensional crystals

Kazuhiro Abe, Kazutoshi Tani, Tomohiro Nishizawa, Yoshinori Fujiyoshi

Department of Biophysics, Faculty of Science, Kyoto University, Japan

P-040

Conformational change of Arg123 in chloride uptake of light-driven chloride pump Halorhodopsin

Megumi Kubo[1], Takashi Kikukawa[2], Seiji Miyauchi[3], Masakatsu Kamiya[1], Tomoyasu Aizawa[1], Keiichi Kawano[4], Naoki Kamo[1], Makoto Demura[1]

[1]Faculty of Advanced Life Science, Hokkaido University, [2]Creative Research Initiative Sosei, Hokkaido University, [3]Faculty of Pharmaceutical Science, Matsuyama University, [4]Graduate School of Science, Hokkaido University, Japan

P-041

Functional expression of NMDA subtype glutamate receptor by a novel bi-cistronic baculovirus expressoin vector

Tzong-Yuan Wu

Department of Bioscience Technology, Chung Yuan Christian University, Taiwan

P-042

Protein-free selective retrieval of crosslinked peptides for mass spectrometric analysis of protein complexes

Ruth Hogue Angeletti, Funing Yan, Fa-Yun Che, Dmitrij Rykunov, Carlos Madrid, Edward Nieves, Louis M. Weiss, Andras Fiser

Biodefense Proteomics Research Center, Albert Einstein College of Medicine, USA

P-043

Investigating the *Plasmodium falciparum* Hsp90 chaperone complex, a potential antimalarial drug target

Chun Song Chua, Cher Siong Gan, Tiow Suan Sim

Department of Microbiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

P-044

Dynamic structural analysis of the electron transfer complex between Cytochrome *c* and Cytochrome *c* Oxidase revealed by ^{15}N relaxation measurements

Koichi Sakamoto[1], Masakatsu Kamiya[2], Kyoko Shinzawa-Itoh[3], Tomoyasu Aizawa[2], Makoto Demura[2], Keiichi Kawano[1], Shinya Yoshikawa[3], Koichiro Ishimori[1]

[1]Department of Chemistry, Graduate School of Science, Hokkaido University, [2]Division of Molecular Life Science, Graduate School of Life Science, Hokkaido University, [3]Department of Life Science, Graduate School of Life Science, University of Hyogo, Japan

P-045

The elongated domains of L4 and L22 and their role in the ribosome function

Aikaterini Tsagkalia[1], Knud Nierhaus[2], Lasse Lindahl[3], Janice Zengel[3], **Theodora Choli-Papadopoulou**[1]
[1]School of Chemistry, Laboratory of Biochemisrty, Aristotle University of Thessaloniki, [2]Max-Planck Institut for Molecular genetics, Berlin, Germany, [3]University of Maryland, Baltimore Country, USA

P-046

Phosphorylation of S5 ribosomal protein from mouse by Casein Kinase II is essential for its cellular traffic

Helen Papachristou[1], Christina Matragou[1], Zoe Karetsou[2], Thomais Papamarcaki[2], **Theodora Choli-Papadopoulou**[1]
[1]School of Chemistry, Laboratory of Biochemisrty, Aristotle University of Thessaloniki, [2]Laboratory of Biological Chemistry, Medical School, University of Ioannina, Greece

P-47 (Y-08)

An alternative splicing variant of the PPM1 protein phosphatase PPM1D

Yoshiro Chuman, Wataru Kurihashi, Yohei Mizukami, Takehiro Nashimoto, Hiroaki Yagi, Kazuyasu Sakaguchi
Department of Chemistry, Faculty of Science, Hokkaido University, Japan

P-048

The antimicrobial peptide LL-37 inhibits biofilm formation of uropathogenic *E. coli*

Ylva Kai-Larsen[1], Milan Chromek[2], Asa Holm[3], Petra Luthje[3], Xiaoda Wang[4], Jan Johansson[5], Ute Romling[4], Annelie Brauner[3], Birgitta Agerberth[1]
[1]Department of Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm, Sweden, [2]Department of Paediatrics, Karolinska University Hospital Huddinge, Stockholm, Sweden, [3]Division of Clinical Microbiology, Department of Microbiology, Tumor and Cell Biology, Karolinska University Hospital and Karolinska Institute, Stockholm, Sweden, [4]Department of Microbiology, Tumor and Cell Biology, Karolinska Institute, Stockholm, Sweden, [5]Department of Anatomy, Physiology and Biochemistry, Swedish University of Agricultural Sciences, the Biomedical Centre, Uppsala, Sweden

P-049 (Y-04)

New approaches for developing therapeutic drugs against neutrophil activation by HPNAP from Helicobacter pylori

Filippos Kottakis[1], Georgios Papadopoulos[3], Christina Befani[1], **Antonios Asiminas**[1], Georg Koliakos[2], Theodora Choli-Papadopoulou[1]
[1]Aristotle University of Thessaloniki, School of Chemistry, Laboratory of Biochemistry, [2]Aristotle University of Thessaloniki, Medical School , Laboratory of Biological Chemistry, [3]Department of Biochemistry and Biotechnology, University of Thessaly, Larissa, Greece

P-050 (Y-07)

Toll-like receptors 2 and 4 can be activated by vEP protease to induce an inflammatory response

Jung Eun Park, Nam Hee Lee, Jong Woo Park, Jae Yeong Park, Jung Sup Lee
Department of Biotechnology, BK21 Research Team for Protein Activity Control, and Research Center for Proteineous Materials, Chosun University, Gwangju 501-759, Republic of Korea

P-051

A serine protease secreted by Staphylococcus aureus evokes a vascular permeability accompanied with production of pro-inflammatory cytokines

Jong Woo Park, Eu-Ri Jo, Jung Eun Park, Jung Sup Lee
Department of Biotechnology, BK21 Research Team for Protein Activity Control, and Research Center for Proteineous Materials, Chosun University, Gwangju 501-759, Republic of Korea

P-052

Isolation and characterization of a fibrinogenolytic enzyme from Macrovipera mauntanica snake venom

Eun Hee Lee, Jung Eun Park, Jung Sup Lee
Department of Biotechnology, BK21 Research Team for Protein Activity Control, and Research Center for Proteineous Materials, Chosun University, Gwangju 501-759, Republic of Korea

P-053

Elucidating potential phospho-regulatory sites in Pfnek3, a *Plasmodium falciparum* protein kinase, to rationalize its cellular activation

Huiyu Low, Tiow Suan Sim
Department of Microbiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

P-054 (Y-06)

Alteration of *Streptomyces clavuligerus* deacetoxycephalosporin C synthase substrate binding pocket to accommodate penicillins with aromatic side chains

Kian Sim Goo, Tiow-Suan Sim
Department of Microbiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

P-055

Role of C-terminal region of chaperonin GroEL: Identification of the functionally critical amino acid segment

Yasushi Kawata[1,2], Kodai Machida[2], Akane Kono-Okada[1], Kunihiro Hongo[1,2], Tomohiro Mizobata[1, 2]
[1]Department of Chemistry and Biotechnology, Graduate School of Engineering, Tottori University, Japan, [2]Department of Biomedical Science, Graduate School of Medical Science, Tottori University, Japan

P-056

Study on structural and functional roles of the C-terminal α-helix of calmodulin

Chihiro Kitagawa[1], Satoshi Nagaya[1], Kumiko Nakamura[1], Ryosuke Doi[1], Akiko Nakatomi[2], Michio Yazawa[2]
[1]Department of Chemistry, Graduate School of Science, Hokkaido University, [2]Division of Cellular Life Scisence, Faculty of Advanced Life Science, Hokkaido University, Japan

P-057

Down-regulation of boar PDI-P5 having thiol reductase and antichaperone activities during the last stage of epididymal sperm maturation

Kuniko Akama[1,2], Tomoe Horikoshi[1], Atsushi Sugiyama[1], Aoi Akitsu[3], Nobuyoshi Niwa[3], Atsushi Intoh[3], Michiko Sugaya[2], Kazuo Takei[3], Noriaki Imaizumi[3], Rena Matsumoto[2,4], Hitoshi Iwahashi[2, 5], Shin-ichi Kashiwabara[6], Tadashi Baba[6], Megumi Nakamura[7], Tosifusa Toda[7]
[1]Department of Chemistry, Graduate School of Science, Chiba University, [2]Graduate School of Science and Technology, Chiba University, [3]Department of Chemistry, Faculty of Science, Chiba University, [4]International Patent Organism Depositary, National Institute of Advanced Industrial Science and Technology, [5]Health Engineering Research Center, National Institute of Advanced Industrial Science and Technology, [6]Graduate School of Life and Environmental Sciences, University of Tsukuba, [7]Research Team for Molecular Biomarkers, Tokyo Metropolitan Institute of Gerontology, Japan

P-058 (Y-09)

Landscaping enzymatic reactions of oxidoreductases by a self-organizing neural network

Keisuke Ueno, Katsuhiko Mineta, Toshinori Endo
Graduate School of Information Science and Technology, Hokkaido University, Japan

P-059

Transmembrane peptides are useful tools for study of membrane proteins function

Daisuke Sugiyama[1], Satoshi Osada[1], Ryo Hayashi[1], Hiroshi Yamaguchi[1], Masoud Jelokhani-Niaraki[2], Ichiro Fujita[3], Hiroaki Kodama[1]
[1]Department of Chemistry, Faculty of Science and Engineering, Saga University, [2]Department of Chemistry, Wilfrid Laurier University, [3]Department of Pediatrics, Faculty of Medicine, Saga University, Japan

P-060

Stoichiometry and interaction of proteins in the wedge assembly pathway of bacteriophage T4

Moh Lan Yap, Shuji Kanamaru, Fumio Arisaka
Department of Biomolecular Process Engineering, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, Japan

P-061

The novel methodology to tune the protein-protein interaction by ligand.

Kumiko Suzuki, Toshihisa Mizuno, Toshiki Tanaka
Department of Material Science, Graduate School of Engineering, Nagoya Institute of Technology, Japan

P-062

Structure and function of calmodulin immobilized on solid substrate studied by in situ ATR-IR and SFG spectroscopies -Ca^{2+} induced binding of mastoparan and structure change of interfacial water-

Tatsuhiko Adachi[1], Hidenori Noguchi[1], Akiko Nakatomi[2], Michio Yazawa[2], Kohei Uosaki[1]
[1]Department of Chemistry, Faculty of Science, Hokkaido University, [2]Division of Cellular Life Science, Graduate School of Life Science, Hokkaido University, Japan

P-063

Dynamic assembly properties of nonmuscle myosin II isoforms revealed by fluorescence cross-correlation spectroscopy

Mariko Mitsuhashi[1], Hiroshi Sakata[2], Masataka Kinjo[2], **Masayuki Takahashi**[1], Michio Yazawa[2]
[1]Department of Chemistry, Faculty of Science, Hokkaido University, [2]Division of Cellular Life Science, Faculty of Advanced Life Science, Hokkaido University, Japan

P-064

Surface plasmon resonance and mass spectrometric analysis of protein interaction in heme catabolism

Hiroshi Sakamoto[1], Yuichiro Higashimoto[2], Masakazu Sugishima[2], Masato Noguchi[2]
[1]Department of Bioscience and Bioinformatics, Kyushu Institute of Technology, [2]Department of Medical Biochemistry, Kurume University School of Medicine, Japan

P-065

Prion protein structural distinctiveness characterized by octapeptide tandem repeat

Yuji Horiuchi, Eriko Hattori, Satoru Yokotani, Ayami Matsushima, Takeru Nose, Yasuyuki Shimohigashi
Laboratory of Structure-Function Biochemistry, Department of Chemistry, The Research-Education Centre of Risk Science, Faculty and Graduate School of Sciences, Kyushu University, Fukuoka 812-8581, Japan

P-066

Comprehensive proteomic analyses of human heterochromatin protein 1 (HP1) interacting factors

Ryu-suke Nozawa[1], Koji Nagao[2], Osamu Iwasaki[1], Chikashi Obuse[1]
[1]Faculty of Advanced Life Science Hokkaido University, [2]G0 Cell Unit, Initial Research Project, Okinawa Institute of Science and Technology, Japan

P-067
Ideal BRC peptide sequence for the inhibition of filament formation of human Rad51 protein
Julian Nomme[1], Yuya Asanomi[2], Vinh Tran[1], Kazuyasu Sakaguchi[2], **Masayuki Takahashi**[1]
[1]UMR6204 CNRS & Universite de Nantes, [2]Department of Chemistry, Faculty of Science, Hokkaido University, Japan

P-068 (S-06)
RNA-binding CCCH-type zinc-finger proteins are essential for oocyte maturation in *C. elegans*
Masumi Shimada[2], Hideyoshi Yokosawa[1], **Hiroyuki Kawahara**[2]
[1]Department of Biochemistry, Graduate School of Pharmaceutical Sciences, Hokkaido University, [2]Department of Biological Sciences, Tokyo Metropolitan University, Japan

P-069
Structural basis for dynamical interdomain movement of the selenocysteine-specific elongation factor SelB
Toyoyuki Ose[3], Nicolas Soler[2], Linda Rasubala[1], Kimiko Kuroki[1], Daisuke Kohda[1], Dominique Fourmy[2], Satoko Yoshizawa[2], Katsumi Maenaka[1]
[1]Division of Structural Biology, Medical Institute of Bioregulation, Kyushu University, [2]Laboratoire de Chimie et Biologie Structurales, ICSN-CNRS, [3] Faculty of Life Science, Hokkaido University, Japan

P-070 (Y-05)
Structural and functional analysis of a novel, highly conserved, glomerulus specific protein shisa3
Juha R.M. Ojala[1], Zhijie Xiao[1], Jaakko Patrakka[1], Christer Betsholtz[2], Karl Tryggvason[1]
[1]Department of Medical Biochemistry and Biophysics, Division of Matrix Biology, [2]Vascular Biology, Karolinska Institutet, Sweden

P-071
Quantitative analysis of oligomeric stability and transcriptional activity of tumor suppressor protein p53
Rui Kamada, Tomoko Terai, Takao Nomura, Yoshiro Chuman, Toshiaki Imagawa, Kazuyasu Sakaguchi
Department of Chemistry, Faculty of Science, Hokkaido University, Japan

P-072
A functional region of ELC required for the formation of 10S conformation in smooth muscle myosin
Tsuyoshi Katoh, Takanobu Taniguchi
Department of Biochemistry, Faculty of Medicine, Asahikawa Medical College, Japan

P-073 (Y-03)
Xyloglucan recognition mechanism suggested from the crystal structure of Cel44A
Yu Kitago[1], Shuichi Karita[2], Nobuhisa Watanabe[3], Kazuo Sakka[2], Isao Tanaka[1]
[1]Faculty of Advanced Life Science, Hokkaido University, [2]Major of Sustainable Resource Science, Graduate School of Bioresources, Mie University, [3]Graduate School of Engineering, Nagoya University, Japan

P-074
Elucidation of an unusual and catalytically critical hydrogen bond network in the catalytic center of human glutaminyl cyclase
Kai-Fa Huang[1], Yu-Ruei Wang[1], En-Cheng Chang[1], Tsung-Lin Chou[2], Andrew H.-J. Wang[1]
[1]Institute of Biological Chemistry, Academia Sinica, [2]Institute of Biochemical Science, National Taiwan University, Taiwan

P-075
Analysis of stability for p53 tetramerization domain peptides derived from various species
Takao Nomura, Rui Kamada, Yoshiro Chuman, Kazuyasu Sakaguchi
Department of Chemistry, Faculty of Science, Hokkaido University, Japan

P-076
Site-directly ^{19}F-labeled collagen model peptides
Nozomi Sato[1], Kazuki Kawahara[2], Daisuke Motooka[1], Masamitsu Doi[3], Yuji Nishiuchi[4], Nobuaki Nemoto[5], Takashi Nakazawa[6], Yoshinori Nishi[1], Takuya Yoshida[2], Tadayasu Ohkubo[2], Yuji Kobayashi [1]
[1]Osaka University of Pharmaceutical Sciences, [2]Graduated School of Pharmaceutical Sciences, Osaka University, [3]Wakayama National Collage of Technology, [4]Peptide Institute Inc, [5]JEOL LTD, [6] Department of Chemistry, Nara women's University, Japan

P-077
Effects of thermodynamic stability on expression level of p53 tetramerization domain peptides in *E.coli*
Hiromitsu Miyazaki, Rui Kamada, Takao Nomura, Yoshiro Chuman, Toshiaki Imagawa, Kazuyasu Sakaguchi
Department of Chemistry, Faculty of Science, Hokkaido University, Japan

P-078 (Y-01)
Structural flexibility of human nuclear receptor ERRγ to adopt endocrine disruptor bisphenol A and its derivatives
Ayami Matsushima[1], Hiroyuki Okada[1], Xiaohui Liu[1], Takatoshi Tokunaga[1], Takamasa Teramoto[2], Yoshimitsu Kakuta[2], Yasuyuki Shimohigash [1]
[1]Department of Chemistry, The Research-Education Centre of Risk Science, Faculty and Graduate School of Sciences, Kyushu University, [2]Laboratory of Biochemistry, Department of Agricultural Chemistry, Faculty and Graduate School of Agriculture, Kyushu University, Japan

P-079

Diversity of bisphenol A-specific nuclear receptor ERR-γ due to the alternative pre-mRNA splicing

Yukimasa Takeda[1], Xiaohui Liu[1], Miho Sumiyoshi[2], Ayami Matsushima[1], Miki Shimohigashi[2], **Yasuyuki Shimohigashi**[1]
[1]Department of Chemistry, The Research-Education Centre of Risk Science, Faculty of Sciences, Kyushu University, [2]Division of Biology, Faculty of Science, Fukuoka University, Japan

P-080

A unique bimodal expression profile of intron-retained PERIOD splicing variants in the fruit fly *Drosophila melanogaster*

Yukimasa Takeda[1], Miho Sumiyoshi[2], Ayami Matsushima[1], Miki Shimohigashi[2], **Yasuyuki Shimohigashi**[1]
[1]Department of Chemistry, Faculty of Sciences, Kyushu University, [2]Division of Biology, Faculty of Science, Fukuoka University, Japan

P-081

Intrinsically disordered structure of the C-terminal region in the *Helicobacter pylori* oncoprotein CagA

Takeru Hayashi[1], Masafumi Horio[1], Hideaki Higashi[1], Hiroyuki Kumeta[2], Fuyuhiko Inagaki[2], Masanori Hatakeyama[1]
[1]Institute for Genetic Medicine, Hokkaido University, [2]Graduate School of Pharmaceutical Science, Hokkaido University, Japan

P-082

Heme transfer mechanism from heme chaperone protein, CcmE, to apocytochrome c

Takeshi Uchida, Koichiro Ishimori
Department of Chemistry, Faculty of Science, Hokkaido University, Japan

P-083

New potential therapeutic targets for ovarian clear cell carcinoma

Noriaki Arakawa[1], Yusuke Masuishi[1], Yuko Yamanaka[1], Hiroshi Kawasaki[1], Etsuko Miyagi[2], Fumiki Hirahara[2], Hisashi Hirano[1]
[1]Supermolecular Biology, International Graduate School of Arts and Sciences, Yokohama City University, [2]Department of Obstetrics and Gynecology, Yokohama City University School of Medicine, Japan

P-084

Tumor intracellular signal-targeting by application of specific substrate peptide for protein kinase Cα

Daisuke Asai[1], Jeong -H. Kang[2], Riki Toita[3], Tetsuro Tomiyama[3], Takeshi Mori[2,4], Takuro Niidome[2,3,4,5], Hideki Nakashima[1], Yoshiki Katayama[2,3,4]
[1]Department of Microbiology, St. Marianna University School of Medicine, [2]Department of Applied Chemistry, Faculty of Engineering, Kyushu University, [3]Graduate School of Systems Life Sciences, Kyushu University, [4]Center for Future Chemistry, Kyushu University, [5]PRESTO, J Japan Science and Technology Agency, Japan

P-085

The effect of CDX1 transcription factor on human gastric epithelial cells

Yumiko Fujii[1], Daisuke Sasaya[1], Naoko Kamimura[2], Hiroyuki Aburatani[2], Masanori Hatakeyama[1]
[1]Inst. Genetic Medicine, Hokkaido Univ., [2]RCAST, Univ. Tokyo, Japan

P-086

Binding of mouse prion protein to heparin

Kenta Teruya[1], Masahiro Wakao[3], Tomoaki Nishimura[2], Tomohiro Kimura[1], Yuji Sakasegawa[1], Yasuo Suda[3], Katsumi Doh-ura[1]
[1]Department of Prion Research, Graduate School of Medicine, Tohoku University, [2]SUDx-Biotech Corp., [3]Department of Nanostructure and Advanced Materials, Graduate School of Science and Engineering, Kagoshima University, Japan

P-087

Proteomic analysis of serum from diabetic LEA/Sendai rats

Eri Takahashi[1], Tadashi Okamura[3], Kohei Ikari[2], Hisashi Hirano[1], Kazuki Yasuda[2], Yasushi Kaburagi[2]
[1]Supramolecular Biology, International Graduate School of Arts and Sciences, Yokohama City University, [2]Department of Metabolic Disorder, Research Institute, International Medical Center of Japan, [3]Department of Infectious Diseases, Research Institute, International Medical Center of Japan, Japan

P-088

Epitope analysis of kiwi fruit allergen actinidin

Tomohiro Nanri[1], Satomi Sugiyama[1], Yuri Kosuge[2], Yasuharu Itagaki[3], Hisashi Hirano[1]
[1]Yokohama City University International Graduate School of Arts and Sciences, [2]Nihon university Colleage of Biosourse Sciences, [3]Kanagawa Academy of Science and Technology, Japan

P-089 (S-07)

Analysis of specific proteins related to chemotherapy sensitivities in gliomas by assembled proteomic strategies.

Norie Araki[1], Anthony Wilson[1], Takashi Morikawa[1], Nobuyuki Tsubota[2], Megumi Nagayama[1], Daiki Kobayashi[1], Masayo Wilson-Morifuji[1], Uichi Midorikawa[1], Hideo Nakamura[2], Junichi Kuratsu [2]
[1]Department of Tumor Genetics and Biology, Graduate School of Medical Sciences, Kumamoto University, [2]Department of Neurosurgery, Graduate School of Medical Sciences, Kumamoto University, Japan

P-090

Identification and characterization of cholangiocarcinoma-associated antigens recognized by specific monoclonal antibodies.

Atit Silsirivanit[1], Sopit Wongkham[1], Chaisiri Wongkham[1], Chawalit Pairojkul[2], Vajarabhongsa Bhudhisawasdi[3], Kazuhiko Kuwahara[4], Nobuo Sakaguchi[4], Anthony Wilson[5], Megumi Nagayama[5], Daiki Kobayashi[5], Masayo Morifuji Wilson[5], Norie Araki[5]

[1]Department of Biochemistry, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand, [2]Department of Pathology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand, [3]Department of Surgery, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand, [4]Department of Immunology, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan, [5]Department of Tumor Genetics and Biology, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan

P-091

Study of molecular mechanisms in the development of highly metastatic human tongue cancer cells using combined differential transcriptomic and proteomic analysis

Wilson-Morifuji Masayo, Wilson Anthony, Daiki Kobayashi, Takashi Morikawa, Masayuki Tsubota, Norie Araki

Dept. Tumor Genet. Biol., Grad. Sch. Med. Sci., Kumamoto Univ., Japan

P-092

Discovery and validation of the protein biomarkers for tumor diagnosis: Differential analyses of the liquid chromatography/tandem mass spectrometry profiles from the tissue proteomes

Takao Kawakami[1], Atsushi Ogiwara[2], Kazuya Wada[2], Takashi Hirano[1], Harubumi Kato[3], Norihiko Ikeda[1]

[1]Department of Surgery, Tokyo Medical University, [2]Biomarker Mining Team, Medical ProteoScope Company, [3]International University of Health and Welfare Graduate School, Japan

P-093

Development of the basic strategy for the comparative analysis of serum proteomes and its application in the discovery of hepatic injury biomarkers

Yoshio Kodera[3], Yusuke Kawashima[1], Tomoyuki Fukuno[1], Masahiro Maruhashi[1], Hiroki Takahashi[1], Takashi Matsui[1], Hiroyoshi Komatsu[4], Masanori Seimiya[5], Takeshi Tomonaga[5], Fumio Nomura[5], Tadakazu Maeda[1]

[1]Department of Physics, School of Science, Kitasato University, [2]Center for Disease Proteomics, School of Science, Kitasato University, [3]Clinical Proteomics Research Center, Chiba University Hospital, [4]Department of Clinical Laboratory Medicine, Faculty of Health Science Technology, Bunkyo Gakuin University, [5]Department of Molecular Diagnosis (F8), Graduate School of Medicine, Chiba University, Japan

P-094

Novel chemical inhibitors for p53-inducible protein phosphatase PPM1D

Hiroaki Yagi, Yoshiro Chuman, Fumihiko Yoshimura, Keiji Tanino, Kazuyasu Sakaguchi

Department of Chemistry, Faculty of Science, Hokkaido University, Japan

P-095

Characterization of cisplatin-resistant gastric cancer cell lines by quantitative protein kinetic analysis using high-density 'reverse-phase' protein lysate microarrays

Hironobu Noda, Satoshi Nishizuka, Kazushige Ishida, Go Wakabayashi

Molecular Therapeutics Laboratory, Department of Surgery, Iwate Medical University School of Medicine, Morioka, Japan

P-096

Analysis of molecular network kinetics induced by anticancer drugs for the identification of crucial molecular targets

Kazushige Ishida, Satoshi Nishizuka, Hironobu Noda, Go Wakabayashi

Molecular Therapeutics Laboratory, Department of Surgery, Iwate Medical University School of Medicine, Morioka, Japan

Lectures

KL-1

PL-1

L-01 ~ L-21

KL-1

Degradomics: the proteolysis of cell death

James A. Wells

Departments of Pharmaceutical Chemistry and Cellular & Molecular Pharmacology, University of California at San Francisco

Apoptosis, or programmed cell death, represents an ultimate fate decision in cell biology. This process is critical for cellular differentiation and remodeling of tissues, and for anti-viral and anti-tumor defense. The study of apoptotic pathways has important ramifications for determining what is critical for cellular homeostasis, and for the development of potential anti-cancer therapeutics. A distinct molecular feature of apoptosis is the widespread but controlled cellular proteolysis, that is predominantly mediated by eight members of the caspase family of cysteine proteases. These enzymes are like demolition experts that cleave protein targets critical for cellular life. We have developed a robust proteomic method for global profiling of proteolysis (degradomics) in cells. Key to this is a new method that permits selective labeling and enrichment for the protein N-termini created as a result of proteolysis. Using this approach we have already identified >300 caspase substrates from Jurkat cells that were induced to undergo apoptosis by treatment with the chemotherapeutic agent etoposide. More than 80% of these proteins have not been reported before. These proteins fall into a wide range of functional classes, and reveal much about the molecular components, logic, and timed sequence of events that drive a cell from life to death. We believe these proteomic appproach will be useful for characterizing the proteolysis of apoptosis induced by various agents or in different cell types, and will be generally useful for dissecting protease signaling pathways.

James A. Wells

Professor

Departments of Pharmaceutical Chemistry and
Cellular & Molecular Pharmacology,
University of California at San Francisco, USA

jim.wells@ucsf.edu

TEL: +1-415-514-4498 FAX: +1-415-514-4507

Education

University of California, Berkeley, CA B.A. 1973 Biochemistry
Washington State University Ph.D. 1979 Biochemistry
Washington State University Postdoc 1979-1980 Chemistry
Stanford University Medical School Postdoc 1980-1982 Biochemistry

Positions & Employment

1982-1986	Scientist, Genentech, Inc., Dept. of Protein Engineering
1986-1989	Senior Scientist, Genentech, Inc., Dept. of Protein Engineering
1989-1998	Staff Scientist, Genentech, Inc., Dept. of Protein Engineering
1988-2005	Adjunct Assistant, Associate, and Full Professor, University of California, San Francisco, Dept of Pharmaceutical Chemistry
1998-2005	President and Chief Scientific Officer, Sunesis Pharmaceuticals
2005-present	Harry Wm. and Diana V. Hind Professor in Pharmaceutical Sciences, University of California, San Francisco
2005-present	Director, Small Molecule Discovery Center, University of California, San Francisco

Other Experience and Professional Memberships

1994-2003	Organizer or Co-organizer of various scientific meetings (e.g., 2003 Keystone Drug Discovery Meeting; 2000 Protein Society meeting, 1999 ASBMB meeting, 1994 Peptides in Biology GRC)
1989-present	Editorial Board service on scientific journals (Proteins, Protein Engineering, Protein Science, Chemistry & Biology, Journal Molecular Biology, Trends in Biotechnology, PNAS)
1994-1998	Member of the Executive Council of Protein Society
2005-2007	Chair of NIH study section on Molecular Libraries Screening Centers Network (MLSCN)
2005-present	Member of the Executive Committee for QB3 at UCSF
2006	Member UCSF Cancer Center
2007-present	Member of the Executive Council ASBMB

Honors (selected from a total of 18)

1979-1981	Damon M. Runyon - Walter Winchell Postdoctoral Fellowship
1990	Pfizer Award (given by the American Chemical Society for achievements in enzyme chemistry
1998	Recipient of the Christian B. Anfinson Award presented by the Protein Society
1998	Recipient of the Vincent du Vignead Award given by the American Peptide Society
1999	Elected Member to the National Academy of Sciences
2003	Recipient of the Hans Neurath Award given by the Protein Society
2006	Perlman Lecture Award of the ACS Biotechnology Division
2006	Paul Janssen Prize in Adv. Biotech and Medicine

PL-1

Proteomic strategies for biomarker discovery and validation

Stephen R. Pennington

Proteome Research Centre, UCD Conway Institute, University College Dublin, Ireland

Dramatic advances in protein-based discovery and validation have been made in recent years. Two dimensional gel electrophoresis (2-DE) and liquid chromatography (LC) based protein separations combined with mass spectrometry (MS) based protein and peptide characterisation remain the benchmarks for discovery studies in which the functional role of proteins is inferred from relative changes in their expression, localisation and/or modification. Both separation platforms (2-DE and LC) offer relative advantages and disadvantages; but both have been dramatically improved by the availability of a range of labelling technologies which have impacted on multiplexing (running several samples simultaneously), software development and differential expression analysis. Examples of the application of both approaches in biomedical discovery projects will be presented.

At present one of the biggest challenges in proteomics remains in the capacity to rapidly validate quantitatively changes in protein expression that have been discovered by existing and emerging comparative expression platforms for appropriate selection of biomarkers for further development. To date, such validation is undertaken largely (but not exclusively) by antibody based approaches including Western blotting, ELISA and IHC (including IHC on tissue microarrays) and again, examples of effective validation of proteomics discoveries by a range of conventional strategies will be presented. However, despite major efforts to generate proteome-scale panels of antibodies these remain relatively slow and require extensive validation of each antibody itself to establish its specificity and utility in different assay formats and are difficult to multiplex. The combination of multiple reaction monitoring (MRM) through application of triple quad linear ion trap mass spectrometry, chemistry and software has provided the opportunity to rapidly develop fully quantitative multiplexed assays of protein expression and post-translation modification that are highly specific and sensitive.

Here we will present our on-going efforts to investigate MRM based measurements of protein expression drawing on efforts to develop more effective and earlier biomarkers of drug toxicity. Toxicity issues remain a significant problem for drug development efforts. Specifically, current early biomarkers of toxicity are insufficient as demonstrated by the high failure rate of candidate therapeutics from safety and toxicity issues. The liver is an important site for metabolism of drugs, environmental pollutants, carcinogens, etc, and the cytochrome P450 protein superfamily of mono-oxygenases is the major phase I enzyme system responsible for metabolism of these molecules in this organ. The four major families of P450s involved in drug metabolism comprise ~25 different isoforms, with different substrate specificities and which are inducible by different drugs or chemicals. Because of the role of cytochrome P450 enzymes in determining drug disposition and toxicity, a detailed knowledge of the various protein isoform preferences of new chemical entities is extremely important and is also a regulatory requirement for pharmaceutical companies engaged in development of novel drug therapies. To understand changes in expression of the P450 proteins and other liver proteins in response to drug treatment we have developed an MRM strategy to simultaneously and quantitatively measure the expression of multiple major P450 isoforms. This approach is widely (if not universally) applicable to the measurement of the expression of the other proteins. More recently, within a large scale consortium, we are currently investigating integrated 'omics strategies for new toxicity marker discovery with subsequent validation of their expression by absolute protein quantification using peptide multiple reaction monitoring (MRM).MRM based strategies, whilst very powerful, do have some limitations and these will be discussed in the context of our goal to develop more clinically relevant and useful biomarkers.

References

Kitteringham NR, Powell H, Jenkins RE, Hamlett J, Lovatt C, Elsby R, Henderson CJ, Wolf CR, Pennington SR, Park BK. (2003) Protein expression profiling of glutathione S-transferase pi null mice as a strategy to identify potential markers of resistance to paracetamol-induced toxicity in the liver. *Proteomics* **3**:191-207

Jenkins RE, Kitteringham NR, Hunter CL, Webb S, Hunt TJ, Elsby R, Watson RB, Williams D, Pennington SR, Park BK. (2006) Relative and absolute quantitative expression profiling of cytochromes P450 using isotope-coded affinity tags. *Proteomics* **6**:1934-47

Stephen R. Pennington

Professor

Proteome Research Centre, UCD Conway Institute,
University College Dublin, Ireland

stephen.pennington@ucd.ie

TEL: +353-1-716-6784 FAX: +353-1-716-6783

Prof. Stephen R. Pennington graduated from Imperial College of Science and Technology (University of London) with a joint honours degree in Chemistry and Biochemistry (upper second) before completing a PhD in Biochemistry at the University of Cambridge. During his PhD he was awarded an Elmore Medical Research Fellowship. It was during this fellowship that his interests in the regulation of the mammalian cell cycle began, the subject of his research that was continued when he moved the Department of Human Anatomy & Cell Biology at the University of Liverpool to take up a post as a Wellcome Trust funded lecturer. Subsequently he was a University Lecturer and then Senior Lecturer. During this period his research included the use of two-dimensional gel electrophoresis (2-DE) which was subsequently combined with mass spectrometry for protein characterisation. This general strategy in its many applications served as the foundation for the evolution of the exciting field of 'Proteomics'.

He has been active in the practical implementation of collaborative proteomics projects and set up a multi-user proteomics facility at Liverpool that served as a 'prototype' for the facility he established when he moved to the Conway Institute in 2003. His vision for the development of proteomics in the Conway Institute is to use his expertise and experience to develop the Proteomics Research Centre to exploit the very latest state-of-the-art instrumentation to attract highly talented senior proteomics researchers to apply proteomics (in its broadest definition) to a diverse range of research programmes – eminent proteomics/glycoproteomics researchers have since joined the UCD Conway Institute, including Dolores Cahill, Michael Dunn, Giuliano Elia, Pauline Rudd and more recently Matthias Wilm.

A particular interest of his is in the development of clinical translational projects by aligning research results to the clinical setting and in the development of methods for the discovery and subsequent quantitative measurement of proteomic biomarkers.

He is on the editorial board of several journals and from 2008 is the Editor in Chief of the journal 'B*i*OMARKERS'; he regularly reviews manuscripts and grants for a large number of organisations.

L-01

Molecular networks, systems medicine and the mechanism of action of drugs

Giulio Superti-Furga, Lily Remsing-Rix, Keiryn Bennett, Jacques Colinge, Gerhard Duernberger, Uwe Rix, Oliver Hantschel

CeMM Center for Molecular Medicine of the Austrian Academy of Science, Austria

Physiology relies on the concerted action of a number of molecular interactions of gene products and metabolites operationally organized in so-called pathways and in yet larger molecular networks. However, current appreciation of the wiring diagram of these pathways is scanty and drug discovery does not yet make use of the new postgenomic appreciation of physiology and pathophysiology. Through integrated approaches using proteomics as central glue it is possible to obtain physical, functional and knowledge maps of human disease pathways. We have started to map systematically the pathway around Bcr-Abl in leukemia and innate immunity pathways. Moreover, it is feasible to map active chemical compounds (imatinib, nilotinib, bosutinib, dasatininb) on the pathways by identifying the protein interactors of the immobilized compounds. The mode-of-action of drugs can be determined, linked to biological processes by positioning on molecular networks and implemented into novel therapeutic and diagnostic approaches. Protein maps will be annotated with chemical entities to a point where structure-binding relationships will be feasible at a proteome-wide scale and the function of pathways assessable using different chemical genetic agents. Such a systems approach and chemical/protein space databases promise to enable important synergies between different research avenues and inaugurate a truly postgenomic pharmacology era.

Giulio Superti-Furga

Director

CeMM Center for Molecular Medicine of the Austrian Academy of Sciences, Austria

gsuperti@cemm.oeaw.ac.at

TEL: +43-1-40-160-70011 FAX: +43-1-40-160-970000

Dr. Giulio Superti-Furga, Ph.D., is Scientific Director and CEO of the Research Center of Molecular Medicine of the Austrian Academy of Sciences and a visiting professor at the Medical University of Vienna.
An Italian national, he performed his undergraduate and graduate studies in molecular biology at the University of Zurich, Switzerland, at Genentech Inc., South San Francisco, USA and at the Institute for Molecular Pathology in Vienna (I.M.P.), Austria. He has been a post-doctoral fellow and Team Leader at the European Molecular Biology Laboratory (EMBL) until 2004. For several years he has served as professor of Biotechnology at the University of Bologna. In 2000, he co-founded the biotech company Cellzome Inc., where he has been Scientific Director and responsible for the Heidelberg research site.
His most significant scientific contributions are the elucidation of basic regulatory mechanisms of tyrosine kinases in human cancers and the discovery of fundamental organization principles of the proteome of higher organisms. Dr. Giulio Superti-Furga is a member of the Austrian Academy of Sciences and of the German Academy of Sciences Leopoldina.

L-02

Chemical genomics of bioactive natural products based on the fission yeast ORFeome

Minoru Yoshida

Chemical Genomics Research Group/Chemical Genetics Laboratory, RIKEN Advanced Science Institute, Japan

Identification of the cellular target of small molecules with interesting biological properties is a major challenge in the development of the small molecules as biological tools and therapeutics. Recently, much attention has been drawn to chemical genetic approach to the cellular target molecules for the active compounds. Mutations, which serve as the genetic determinants responsible for particular phenotypes in classical genetics, are replaced by small-molecule inhibitors in chemical genetics. We have identified histone deacetylase, CRM1/exportin 1, a spliceosome complex component SF3b, etc., as such the specific targets for novel anticancer natural products by the chemical genetic approach. I will present evidence for the importance of these target proteins in a variety of biological processes such as epigenetic control and correct gene expression by splicing. However, it is sometimes difficult to determine the molecular targets using their physical interaction, due to low affinity of the drug to the target and the decrease or even loss of activity by the chemical modifications. Nowadays, thanks to a wealth of genomic information, systematic, genome-wide screening of genetic interaction has become available for drug target discovery and understanding the mechanism of action of bioactive compounds. We recently constructed a whole ORF library (ORFeome) of fission yeast and performed a variety of reverse proteomics including a global analysis of protein subcellular localization (localizome) and a global analysis of protein gel mobility (mobilitome). In addition to these databases, a global screen for the genes that affect drug sensitivity provided insights into the drug target pathway. The usefulness of ORFeome expression for the drug target identification will be discussed.

Minoru Yoshida

Group Director

Chemical Genomics Research Group/
Chemical Genetics Laboratory,
RIKEN Advanced Science Institute, Japan

yoshidam@riken.jp

TEL: +81-48-467-9516 FAX: +81-46-462-4676

Education

The University of Tokyo (Tokyo, Japan)	B.Sc.	1977-1981	Agricultural Chemistry
The University of Tokyo (Tokyo, Japan)	M.Sc.	1981-1983	Agricultural Chemistry
The University of Tokyo (Tokyo, Japan)	Ph.D.	1983-1986	Agricultural Chemistry

Positions & Employment

1986-1994	Assistant Professor, Department of Biotechnology, The University of Tokyo, Tokyo, Japan
1995-2002	Associate Professor, Department of Biotechnology, The University of Tokyo, Tokyo, Japan
2002-	Chief Scientist, Chemical Genetics Lab, RIKEN, Saitama, Japan
2002-2006	Visiting Professor, Department of Biotechnology, University of Tokyo, Tokyo, Japan
2003-	Visiting Professor, Graduate School of Science and Engineering, Saitama University, Saitama, Japan
2007-	Professor, Department of Biotechnology, University of Tokyo, Tokyo, Japan
2008-	Group Director, Chemical Genomics Research Group, and Chief Scientist, Chemical Genetics Lab, RIKEN Advanced Science Institute, Saitama, Japan

Honors

1991	Japan Society for Bioscience, Biotechnology, and Agrochemistry Award for the Encouragement of Young Scientists
1998	Sumiki-Umezawa Memorial Award, Japan Antibiotics Research Association

L-03

Chemical proteomics-based strategies in drug discovery

Gerard Drewes

Cellzome AG, Meyerhofstrasse 1, D-69117 Heidelberg, Germany

Studies of drug action classically assess biochemical activity in settings which typically contain only the isolated target. Recent technical advances in mass spectrometry-based analysis of proteins have enabled the quantitative analysis of sub-proteomes and entire proteomes, thus initiating a departure from the traditional single gene - single protein - single target paradigm. We will review chemical proteomics-based experimental strategies in kinase drug discovery to quantitatively analyse the interaction of small molecule compounds or drugs with a defined sub-proteome containing hundreds of protein kinases and related proteins. One novel approach is based on 'Kinobeads' ・an affinity resin comprised of a cocktail of immobilized broad spectrum kinase inhibitors - to quantitatively monitor the differential binding of kinases and related nucleotide-binding proteins in the presence and absence of varying concentrations of a lead compound or drug of interest. Differential binding is detected by high throughput and sensitive mass spectroscopy techniques utilizing isobaric tagging reagents (iTRAQ), yielding quantitative and detailed target binding profiles. The method can be applied to the screening of compound libraries and to selectivity profiling of lead compounds directly against their endogenously expressed targets in a range of cell types and tissue lysates. In addition, the method can be used to map drug-induced changes in the phosphorylation state of the captured sub-proteome, enabling the analysis of signalling pathways downstream of target kinases.

Gerard Drewes

Vice President Discovery Research

Cellzome AG, Meyerhofstrasse 1,
D-69117 Heidelberg, Germany

Gerard.Drewes@cellzome.com

TEL: +49-6221-13757-350 FAX: +49-6221-13757-200

Dr. Gerard Drewes, PhD, is Vice President of Discovery Research at Cellzome AG, a biopharmaceutical company, a spin off from the *European Molecular Biology Laboratory* located on the EMBL campus in Heidelberg (Germany). After obtaining his Ph.D. in Chemistry from the University of Heidelberg and the Max-Planck-Institute for Medical Research, Gerard was a postdoctoral fellow and staff scientist in Eckhard Mandelkow's lab at the Max-Planck-Institute for Structural Biology in Hamburg. It was here that Gerard discovered the MARK kinases as targets for the treatment of tau pathology in Alzheimer's disease. For this discovery, he was awarded the Boehringer Research Prize in 1998. Since his Habilitation degree in 1999, Gerard is a a lecturer in Biochemistry at the University of Hamburg, and, before joining Cellzome in 2000, he was a Visiting Scientist in the lab of Sir Paul Nurse at the London Cancer Research Institute. Gerard is the author of more than 40 scientific publications in the field of protein kinases, neurobiochemistry, and proteomics, including articles in *Cell*, *Nature*, and *Nature Biotechnology*. In addition, he holds several patents and patent applications, including several on Cellzome's Kinobeads™ technology.

L-04

Diversity & specificity in protein ubiquitination: the BRCA1/BARD1 paradigm

Devin E. Christensen, Peter S. Brzovic, Catherine Eakin, Dawn M. Wenzel, Kate Stoll,
Rachel E. Klevit

Department of Biochemistry, University of Washington, USA

The general model for protein ubiquitination assigns an E3 ligase the dual role of recruiting and binding both a ubiquitin conjugating enzyme (E2) and a substrate. It is generally appreciated that substrate specificity is determined largely by the E3 and that a given E3 likely targets numerous substrates for modification. The relationship between an E3 and the E2 has received less attention. Yet, the human genome encodes over thirty E2 enzymes, raising the questions "Why are there so many E2s, what specifies the E3s with which they collaborate, and do they perform different functions?" With hundreds of E3s encoded in the genome, it is clear that a given E2 must work with many E3s, but the converse may also be true. The interactions between E2s and E3s are of modest affinity and are not amenable to most conventional biochemical approaches. We devised a "structure-based" yeast two-hybrid strategy to discover which human E2s bind to the breast cancer protein, BRCA1/BARD1. Although both BRCA1 and BARD1 contain a RING motif, neither subunit is capable of binding an E2 on its own; the heterodimeric structure is required. We show that a bait construct that contains the essential region of BARD1 and BRCA1 fused into a single polypeptide recapitulates the previously known E2 interactors and identifies six novel E2 interactions with BRCA1/BARD1. Comparison of the sequences of the ten interacting E2s with non-interacting E2 sequences allowed identification of an alanine residue that is conserved only in the interacting set. Mutation of the relevant alanine in several BRCA1-interacting E2s abrogates both their binding and activity with BRCA1/BARD1, confirming the central role of the alanine as a determinant of specificity. We will discuss general features of E2/E3 interactions that are likely to serve as specificity determinants for other E3 ligases. The E2s that bind to BRCA1 are active in BRCA1/BARD1 autoubiquitination reactions. Depending solely on the E2 used, either mono- or polyubiquitinated BRCA1/BARD1 products are generated. The polyubiquitin chain linkage depends on the E2 as well, with Lys-48 or Lys-63-linked chains forming on BRCA1 with Ube2K and Ubc13/Mms2, respectively. These two chain-building E2s must work in concert with a monoubiquitinating E2, implying a division of labor among human E2s in which certain of them are responsible for transfer of the first ubiquitin to a lysine residue on a substrate while other chain-building E2s transfer subsequent ubiquitin moieties to elongate a chain with a configuration specified by the E2. Not all BRCA1-interacting E2s are capable of modifying a given substrate, suggesting that an E2 also plays a role in substrate specification. We therefore propose an elaboration of the existing paradigm for protein ubiquitination in which an E2/E3 pair specifies substrate identity, the nature of the product, and, therefore, the ultimate fate of the modified protein.

Rachel E. Klevit

Professor

Department of Biochemistry,
University of Washington, *USA*

klevit@u.washington.edu

TEL: +1-206-543-5891 FAX: +1-206-543-8394

Prof. Rachel E. Klevit received her D. Phil. in Chemistry from the University of Oxford where she pursued her graduate research as one of the first female Rhodes Scholars. Her doctoral research was among the earliest applications of NMR spectroscopy to the EF-hand protein calmodulin. After a post-doctoral American Cancer Society fellowship at Duke University School of Medicine, she moved to the University of Washington in 1984 where one of the first 500 MHz NMR spectrometers has just been installed. In 1986, as a member of the Research Faculty in Chemistry, she assigned the NMR spectrum of a bacterial phosphotransfer protein, HPr, by ^{1}H 2D NMR, the largest protein to have its spectrum assigned at the time. Dr. Klevit joined the faculty as Assistant Professor of Biochemistry in the University of Washington School of Medicine in 1986; she became Full Professor of Biochemistry. In 1988, her group solved the first 3D structure of a zinc finger. Prof. Klevit's research focuses on the structure and function of proteins involved in human disease, including cyclic nucleotide phosphodiesterases PDE5 and PDE6, the sensor kinase PhoQ from pathogenic bacteria, and the breast cancer tumor suppressor BRCA1/BARD1. In 2001, Prof. Klevit and colleagues solved the 3D structure of the portion of the heterodimeric RING complex of BRCA1/BARD1 that is responsible for its activity as a ubiquitin E3 ligase. Subsequent research to better understand the function of the BRCA1/BARD1 ligase has led to studies into the general mechanisms of protein ubiquitination. Prof. Klevit has received numerous awards in recognition of her research contributions, including the Margaret Dayhoff Award of the Biophysical Society, the DuPont Young Investigator Award of The Protein Society, and the ICI Pharmaceutical Group Award for Excellence in Chemistry.

L-05

Prenyltransferases as targets for the discovery of new antibiotics

Andrew H.-J. Wang

Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan

Prenyltransferases are involved in many biological pathways; thus they are useful for developing new drugs for various diseases. We have studied by structural analysis the product chain length determinants of several *trans*-type prenyl-transferases, including geranylgeranyl pyrophosphate synthase (GGPPS) and so on. The specificities were determined by the size and depth of the activity site cavity. Large amino acids form the floor to block product further elongation (1). In addition, we solved the structures of yeast GGPPS complexed with several bisphosphonate inhibitors (2). Undecaprenyl diphosphate synthase (UPPS), a *cis*-prenyltransferase, produces C55-UPP via *cis* double-bond addition. UPP is used for peptidoglycan cell-wall biosynthesis in bacteria. Here, bisphosphonates were tested as inhibitors of UPPS. In the UPPS-inhibitor complexes, four distinct binding sites were observed (2). The availability of these structures opens up new avenues for the design of novel inhibitors. Finally, dehydrosqualene synthase (CrtM) from *Staphylococcus aureus*, uses the head-to-head condensation of two farnesyl diphosphates (FPP) to produce the presqualene diphosphate C30 molecule, the precursor of staphyloxanthin, the golden carotenoid pigment which promotes bacterial resistance to reactive oxygen species and host neutrophil-based killing. CrtM, therefore, has been tested as the target to treat infections by methicillin-resistant *S. aureus* (MRSA). We found squalene synthase inhibitors for cholesterol-lowering activity in humans bind to CrtM and block the biosynthesis of staphyloxanthin *in vitro*, resulting in colorless bacteria with increased susceptibility to killing in a mouse infection model (3).

References
1. Chang TH et al. *JBC* **281**:14991-5000 (2006).
2. Guo RT et al. *PNAS* **104**:10022-7 (2007).
3. Liu CI et al. *Science* **319**:1391-4 (2008).

Andrew H.-J. Wang

Vice President

Institute of Biological Chemistry,
Academia Sinica, *Taiwan*

ahjwang@gate.sinica.edu.tw

TEL: +886-2-2788-1981 FAX: +886-2-2788-2043

Education

B.S., Chemistry, National Taiwan University, 1967
M.S., Chemistry, National Taiwan University, 1970
Ph.D., Chemistry, University of Illinois-Urbana, 1974

Positions & Employment

1974-1988	Postdoctoral Research Associate (74-80), Research Scientist (80-82), Principal Research Scientist (82-85), Senior Research Scientist (85-88), Department of Biology, MIT
1-7/81 & 3-4/83	Visiting Scientist, Gorlaeus Lab., Leiden State Univ., The Netherlands (with Prof. J. H. van Boom)
9/87-2/88	Visiting Associate, Division of Chemistry, Caltech (with Prof. P. B. Dervan)
2/95-5/95	Visiting Professor, Department of Biophysical Chem., Nijmegen University (with Prof. C. Hilbers)
1988-2000	Professor of Biophysics, Biochemistry, and Chemistry, Dept. of Cell & Structural Biology, Biochemistry, UIUC
1996-1997	Acting Head, Dept. of Cell & Structural Biology, UIUC
2000-2006	Distinguished Research Fellow and Director, Institute of Biological Chemistry, Academia Sinica
2006-present	Distinguished Research Fellow and Vice President, Academia Sinica

Membership & Honors

1987	Elected member, American Society for Biochemistry & Molecular Biology
1987	Fellow, American Institute of Chemists
1998	Fellow, American Association for the Advancement of Science
2000	Academician, Academia Sinica
2005	Fellow, The Third World Academy of Sciences (TWAS)
2007	Fellow, A-IMBN
2007	Science and Engineering Achievement Award, Taiwanese-American Foundation

L-06

The role of structural disorder in protein-protein interactions

Péter Tompa

Institute of Enzymology, Biological Research Center, Hungarian Academy of Sciences, Hungary

Intrinsically disordered proteins (IDPs) have received increased attention lately, as it has become clear that the lack of a stable 3D structure is prevalent in the proteomes of higher organisms. Structural disorder is particularly prevalent in regulatory and signaling proteins, and IDPs often carry out their functions via protein-protein interactions (PPIs). In this work we have addressed the principles of the involvement of disorder in PPIs. We have shown that short linear recognition motifs of proteins tend to fall into locally disordered regions, and such regions provide efficient switches for the rapid evolutionary adaptation of interaction networks. The actual binding regions undergo disorder-to-order transition upon binding and adopt well-defined structures in the cognate complexes. Analysis of the secondary structural preferences of these regions has led to the unexpected finding that IDPs have a strong preference for their structures attained in the bound state, which suggests that their binding motifs might presage their final, partner-induced conformations. This fine balance between order and disorder is probably indicative of the thermodynamic and kinetic fine-tuning of recognition by IDPs, which is in line with their preferential functioning in interaction networks. Our studies on PPI networks have shown that disorder is higher in proteins with multiple interactions (hubs proteins) than in proteins with a few interactions. We also have studied atomic details of the interfaces, which suggest that IDPs use a unique strategy for recognition. They present a much higher proportion of their residues for interaction than their globular counterparts, and they tend to expose their hydrophobic residues for effective interaction with their partners. Overall, these and other observations corroborate our premise that involvement in rapid and regulated PPIs has been a key element in the evolutionary advance of protein disorder.

Péter Tompa

Professor

Institute of Enzymology, Biological Research Center,
Hungarian Academy of Sciences, Hungary

tompa@enzim.hu

TEL: +361-279-3143 FAX: +361-466-5465

Prof. Péter Tompa graduated in 1983 in organic chemistry from the University of Eötvös Loránd, Budapest and joined the Institute of Enzymology of the Hungarian Academy of Sciences that year. He has been working there ever since, as a doctorate student until 1990, a postdoctoral fellow until 1998, senior research fellow until 2004, and a scientific advisor from 2004. On sabbatical leaves, he worked in the laboratory of Prof. Sidney Bernhard (University of Oregon), Prof. Koichi Suzuki (University of Tokyo), Prof. Richard Kriwacki (St. Jude Children's Hospital, Memphis, USA), Prof. Jesus Avila (Universidad Autonoma, Madrid, Spain), Prof. Joel Sussman (Weizmann Institute of Science, Rehovot, Israel), and Prof Yun Tang (ECUST University, Shanghai, China) for a total of three years. He received his PhD in 1991, and DSc degree in 2006. He is currently the deputy director of the Institute of Enzymology, and the chair of the Molecular Biology Panel of the Hungarian Scientific Research Fund.

His research initially focused on the transient interactions of soluble glycolytic and TCA-cycle enzymes, which may be regarded as a conceptual prelude to current proteomic initiatives aimed at exploring the interactome of various organisms. Subsequently, his interest turned to the structure-function relationship of the calpain-calpastatin system, its involvement in neuronal plasticity and also some related issues, such as the regulation of cytoskeleton by microtubule-associated protein 2 (MAP2) and the molecular mechanism of prion disease. During this work, he came across the phenomenon of protein disorder, which is at the focus of his current interest. Ha had a basic impact on this developing field, with some key contributions. He authored about 80 papers in international journals.

L-07

GLYCOMICS: Same tools, same questions, different answers

Nicolle H. Packer, Pia H. Jensen, Miyako Nakano, Nandan Deshpande, Albert Lee, Daniel Kolarich

Department of Chemistry and Biomolecular Sciences, Macquarie University, Australia

The analysis of proteins has been revolutionized in the last 10 years so that the high throughput identification of the protein complement of most systems can be accomplished both qualitatively and relatively quantitatively in a matter of weeks if the technology is available. The realization however has now been made that in many cases this knowledge is not sufficient to be able to understand complex biological systems. Protein modifications are manifold and critical for function: one such modification, glycosylation, in itself comprises an analytical science. It has been estimated that over 50% of the cell's proteins have attached carbohydrates and the study of the changes that occur to these sugars, in time and space, glycomics, is now important in its own right. Sugars are expressed on the surface of cells and have been shown to be crucial to the way cells interact in key biological processes such as cancer, fertilisation, inflammation and immunity. Approximately 1% of human genes are used in glycosylation processes, with at least 250 specifically coding for glycosyltransferases. Thirty-four different genetic disorders of glycosylation have so far been identified. The tools of analysis in proteomics and glycomics are the same viz; gels and chromatography, mass spectrometry and bioinformatics, with sugar microarrays available to study interactions. Compared to proteins, there are less building blocks and yet the addition of sugars to the protein confers great variability in structure as well as functionality. Not only does the monosaccharide composition and sequence need to be determined, but also the linkages between the residues, the occupied sites on the protein and the heterogeneity at each site. As a consequence, different methods of sample preparation, analysis and data analysis are required for glycoanalytical research depending on the biological question being asked. Accordingly, it is more difficult to develop, or envisage, a single analytical workflow that will provide all the answers. Different examples of the analysis of the glycosylation of various biologically relevant glycoproteins will be described. These will include methods including enrichment strategies as well as analysis of intact glycoproteins, glycopeptides (including mucin-like O-linked domains) and released N- and O-linked oligosaccharides. The effect of the glycosylation on the biological role, or otherwise, of the glycoprotein will be discussed.

Nicolle H. Packer

Professor

Department of Chemistry and Biomolecular Sciences,
Macquarie University, Australia

nicki.packer@mq.edu.au

TEL: +61-2-9850-8176 FAX: +61-2-9850-6200

Prof. Nicolle Packer is a Professor in the CORE of Biomolecular Frontiers, Department of Chemistry and Biomolecular Sciences, Macquarie University, Sydney, Australia. She has had a career in life sciences research in universities and industry. Her research interests now are in the structure and function of glycans, particularly in their role in cancer and microbial pathogenesis. She co-founded the Australian Proteome Analysis Facility(APAF) and Proteome Systems Limited(PSL), an Australian biotechnology company in which her group developed and applied a platform of glycoanalytical technology and informatics tools. Nicki has published extensively on glycomics research, is a senior editor of *Proteomics,* is a consultant to industry and on the Advisory Board of the Human Disease Glycomics/Proteomics Initiative (HGPI) of HUPO.

L-08

Modification specific proteomics applied to analysis of protein glycosylation

Peter Roepstorff, Martin R. Larsen, Akira Kondo

Department of Biochemistry and Molecular Biology, University of Southern Denmark, Denmark

Post translational modification of proteins is essential for protein function and more than 200 different modifications have been reported. Glycosylation is amongst the most frequent types of modification and also one of the most difficult to analyse due to an extreme heterogeneity of the glycan structures, more than 20 different structures have been found on any given site. It plays a major role in many biological processes including protein folding and secretion, cell recognition, cell-cell interaction, and immune responses. Therefore there is no doubt that detailed studies of the glycome, including the type of glycan structures and their position might be highly relevant for understanding protein function and the cause of diseases. We have in our research group developed a concept, which we term modification specific proteomics. This includes development of a number of methods that allow the selective isolation of glycopeptides to identify which sites in glycoproteins are glycosylated and which glycans are present on the different sites. ZIC-HILIC chromatography has proven to be highly efficient for general glycopeptide enrichment. Specific enrichment of sialic acid containing glycopeptides and GPI-anchors is possible on titanium dioxide columns. The site specific determination of the glycan structures can be performed after reduction of the peptide size by digestion with proteinase K followed by a two step chromatography procedure using micro columns with increasing hydrophobicity followed by tandem mass spectrometry. Quantitative assignment of the amount of glycan occupation on a given site can be achieved by enzymatic deglycosylation in ^{18}O water. The different methods for analysis of glycoproteins will be described and perspectives for further development and for their use in disease studies will be discussed. As an example, analysis of the glycans of beta2-Glycoprotein 1 will be described.

Peter Roepstorff

Professor

Department of Biochemistry and Molecular Biology,
University of Southern Denmark, Denmark

roe@bmb.sdu.dk

TEL: +45-6550-2404 FAX: +45-6593-2661

Education

1965	Diplôme d'études supérieures (physiological chemistry), Faculté des Sciences, Université d'Aix-Marseille.
1966	Graduated in chemical engineering from the Danish Technical University.

Honorary doctor

June 2001	Appointed honorary doctor at the Faculty of Science, Uppsala University, Sweden

Positions & Employment

1966-1974	Research Associate at the Danish Institute of Protein Chemistry.
1974	Assistant Professor at the Dept. of Molecular Biology, Odense University.
1975	Associate Professor at the Dept. of Molecular Biology, Odense University.
1988	Docent at the Department of Molecular Biology, Odense University.
1990-1995	Research Professor under the Ministry of Education, placed at the Dept. of Molecular Biology, Odense University,
1995-2005	Professor in Protein Chemistry, Dept. of Molecular Biology, University of Southern Denmark.
2003-	Adjunct Professor, Faculty of Medicine, University of Bergen.
2005-	Professor emeritus (part time professor), Dept Biochemistry and Molecular Biology, University of Southern Denmark.

Membership & Honors

Kaj Hansens Foundation, "Limprisen" (1968)
"Villum Kann Rasmussens Årslegat for Technical Research" (1989)
Kaj Linderstøm-Lang Gold Medal (2002)
Novo Nordisk Award (2004)
Honorary member of the British Society for Proteome Research (2008)

Member of the Royal Danish Academy of Sciences and Letters (1990)
Member of the Danish Academy of Technical Sciences (1991)

L-09

Discovery of glyco-biomarkers and their biological function using novel glycomics technologies

Hisashi Narimatsu

Research Center for Medical Glycoscience (RCMG), National Institute of Advanced Industrial Science and Technology (AIST), Japan

Association of aberrant glycosylation and various human diseases has long been recognized. However, much of the discovery still depends on serendipity, and biological meanings of these disease-related glycosylation patterns are only gradually uncovered. To facilitate this process by more systematic (i.e., "-omic") approaches, we have initiated a three-tiered project about 7 years ago, with the sponsorship of the New Energy and Industrial Technology Development Organization (NEDO). Glycogene(GG) Project : Better understanding of the molecular basis of glycosylation in humansBy using bioinformatics technologies, we discovered new genes and constructed human glyco-gene library consisting of 183 genes. Knowledge of the substrate specificities of these gene products allowed us to better understand the molecular basis of human glycosylation.Structural Glycomics(SG) Project: Development of highly sensitive glycan structural analysis systemsNext, we started Structural Glycomics (SG) project ('03-'06). Taking full advantage of our glyco-gene library and detailed information regarding their substrate specificities, we developed a glycan library that was then used as standards to develop instruments for glycan structural analysis, such as mass spectrometer-based glycan sequencer and lectin microarray-based glycan profiler.Medical Glycomics (MG) Project: Discovery of disease-related glycosylation patterns and their biological functionsIn 2006, we launched a new project termed Medical Glycomics (MG) project. Our aims in the project are two-folds: (1) development of discovery systems for disease-related glyco-biomarkers, and (2) functional analysis of the disease-associated glycosylation. Armed with our knowledge of human glycosylation, the glycan structural analysis systems, the bioinformatics capability and the databases we have developed over the years, along with animal models of aberrant glycosylation and clinical samples, we are now pursuing this goal.

Hisashi Narimatsu

Director

Research Center for Medical Glycoscience (RCMG),
National Institute of Advanced Industrial Science
and Technology (AIST) , Japan

h.narimatsu@aist.go.jp

TEL: +81-29-861-3200 FAX: +81-29-861-3201

Education

Graduated, March 1974, from Keio University School of Medicine with the degree of M.D.
Graduated, March 1979, from Keio University Postgraduate School of Medicine, and obtained Ph.D.

Positions & Employment

1979-1983	Instructor, Department of Microbiology, Keio University School of Medicine
1983- 1985	Postdoctoral Fellow, Laboratory of Immunology, National Institute of Allergy and Infectious Disease (NIAID), National Institutes of Health (NIH), Bethesda, Maryland, USA
1985- 1986	Postdoctoral Fellow, Laboratory of Pathophysiology, National Cancer Institute (NCI), NIH, Bethesda, Maryland, USA. With Dr. Pradman Qasba.
1986-1986	Instructor, Department of Microbiology, Keio University School of Medicine
1986- 1991	Associate Professor, Instructor, Department of Microbiology, Keio University School of Medicine
1991- 2000	Professor, Division of Cell Biology, Institute of Life Science, Soka University
2000- 2002	Principal Research Scientist, Group Leader of Gene Function Analysis, Institute of Molecular and Cell Biology, National Institute of Advanced Industrial Science and Technology (AIST), Japan
2002- 2006	Group Leader of Glycogene Function Team, Deputy Director of Research Center for Glycoscience, National Institute of Advanced Industrial Science and Technology (AIST), Japan, Professor, Tsukuba University School of Medicine
2006-present	Director of Research Center for Medical Glycoscience, National Institute of Advanced Industrial Science and Technology (AIST), Japan Professor, Tsukuba University School of Medicine

L-10

Strategies for analysis of glycoprotein glycosylation

Hildegard Geyer, **Rudolf Geyer**

Institute of Biochemistry, Faculty of Medicine, Justus-Liebig-University Giessen, Germany

Glycoproteins have been found to play a key role in many biological processes including molecular and cellular recognition, intra- and intercellular signalling, embryonic development, fertilization, immune reactions, inflammation and infection by pathogens such as viruses, bacteria and parasites. In many cases, these properties could be attributed to the carbohydrate chains present. In order to correlate functional features with structural parameters, detailed structural information on the respective carbohydrate moieties is required. The structural analysis of glycoprotein-glycans, however, may be impeded by heterogeneities in monosaccharide composition, sequence, linkage positions and anomeric configurations, oligosaccharide branching and the potential presence of non-carbohydrate substituents.

In recent years, a number of strategies and analytical protocols has been established for carbohydrate epitope screening of glycopeptides and glycoprotein-glycans by multilectin affinity chromatography, oligosaccharide profiling by HPLC or CE of native or reducing end-tagged sugar chains, glycoblotting techniques and, in particular, mass mapping and identification of compositional glycan species by various kinds of mass spectrometric techniques. The large diversity in structures presents problems unique to glycomics and glycoproteomics that cannot be solved by a simple set of tools. Nevertheless the aforementioned work-flows provide versatile platforms for high-throughput analyses as long as sufficient information is available on the enzymes and biosynthetic routes controlling glycoprotein-glycan biosynthesis in the organism from which the glycoprotein has been derived. *De novo* fine structure determination of potentially novel carbohydrate structures expressed, for example, in invertebrates or other sources being less well characterized in this respect, however, is still a challenging task and requires the combined use of different analytical techniques including low-throughput approaches like linkage analysis, enzymatic digestions in conjunction with other analytical procedures and/or high-field NMR spectroscopy. Depending on the quality of information desired, it is therefore necessary to critically evaluate beforehand the analytical strategy to be used.

The aim of this lecture is to review present strategies which have been established as tools for studies in the field of glycomics and glycoproteomics. Simultaneously, inherent limitations of high-throughput, purely mass spectrometric approaches will be addressed and advantages of newly developed software tools for rapid interpretation of mass spectrometry data will be highlighted.

Rudolf Geyer

Professor

Institute of Biochemistry, Faculty of Medicine,
Justus-Liebig-University Giessen, Germany

rudolf.geyer@biochemie.med.uni-giessen.de

TEL: +49-641-994-7400 FAX: +49-641-994-7409

Prof. Rudolf Geyer studied Chemistry at the Technical University of Darmstadt, Germany, and the University of Freiburg, Germany, where he obtained his Ph.D. in Biochemistry in 1977. He moved then to the University of Giessen, Germany, as a research assistant. Since 1990 he is professor of Biochemistry and leader of the Glycobiology Unit at the Institute of Biochemistry of the Faculty of Medicine at the Justus-Liebig-University Giessen. His present research interests are mainly focused on the structure and function of glycoconjugates from parasitic helminths as well as on the carbohydrate structure analysis of mammalian glycoproteins.

L-11

Global mapping of genetic and chemical-genetic networks in yeast

Charles Boone

Banting and Best Department of Medical Research and Department of Molecular Genetics, University of Toronto, Canada

Synthetic Genetic Array (SGA) analysis automates yeast genetics, enabling a number of different large-scale/systematic studies. In one of our major projects, we are attempting to generate the complete synthetic genetic interaction map for yeast cells. This map can be used to define complexes and pathways in the cell, but perhaps more importantly, it adds functional information to the protein-protein interaction map, identifying complexes and pathways that work together and buffer one another. Because a gene deletion mutation provides a model for the effect a target-specific inhibitor, the genetic network provides a key for interpreting chemical-genetic interaction profiles of the complete set of yeast mutants. To expand our genetic and chemical-genetic approaches in yeast, we are constructing a library of all yeast genes in a barcoded vector system, enabling highly parallel approaches for dosage suppression, dosage lethality, cloning by complementation, which can be used to identify drug-resistant mutants that often link compounds to their target pathways.

Charles Boone

Professor

Banting and Best Department of Medical Research
and Department of Molecular Genetics,
University of Toronto, Canada

charlie.boone@utoronto.ca

TEL: +1-416-487-7292 FAX: +1-416-978-8287

Prof. Boone received his Ph.D. in molecular biology in 1989 from McGill University in Montreal, Canada. He then did postdoctoral research in yeast genetics at the Institute of Molecular Biology, University of Oregon, in Eugene. He is currently professor,Canada Research Chair, and Howard Hughes International Research Scholar, at the University of Toronto's Banting and Best Department of Medical Research. His lab focuses on the development and application of functional genomics approaches in yeast. One of his main projects is large-scale synthetic genetic array (SGA) analysis for mapping of the yeast synthetic lethal genetic interaction network. This network serves as a key for defining gene function and interpreting chemical-genetic profiles to link bioactive compounds to their target pathways.

L-12

Proteomic characterization of human pre-ribosome particles

Nobuhiro Takahashi[1], Harunori Yoshikawa[1], Miyuki Kawasaki[1], Toshiya Hayano[2], Mitsuaki Yanagida[3], Sally Fujiyama[1], Yoshio Yamauchi[4], Hideki Ishikawa[1], Toshiaki Isobe[4]

[1]United Graduate School of Agriculture, Tokyo University of Agriculture & Technology,[2]Deartment of Bioscience and Bioinformatics, Ritsumeikan University, [3]Institute for Environmental and Gender Specific Medicine, Juntendo University, [4]Deartment of Chemistry, Tokyo Metropolitan University, Japan

Ribosome biogenesis, which is a process of making ribosome, is essential for cell growth, proliferation, and adaptation, and accounts for up to 80% of the energy consumption of dividing cells, and therefore, disturbances in the ribosome synthesis pathway must be detected and coupled with cell cycle progression to prevent premature cell divisions. Although great advance in understanding the mechanism that links energy status and ribosome biogenesis in response to environmental cues has been made at the level of transcriptional controls of rDNA and mRNAs of ribosomal proteins (1-3), there was very little information concerning the nature of preribosomal ribonucleoprotein (pre--rRNP) complexes as synthetic intermediates of ribosome formed at the post-transcriptional level, in which the assembly of ribosomal proteins onto pre-rRNAs is taken place with a help of a number of trans-acting factors, each of which is associated with the pre-rRNP complex only transiently to perform its action at right place and at right timing. Because of the speed of the trans-acting factors action, it was believed that the isolation of each of the pre-rRNP complexes formed as a synthetic intermediate of ribosome was not possible. In fact, until recently, the identification of many processing and assembly factors in eukaryotes was made on the basis of individual biochemical and genetic studies mostly done in yeast. Proteomic strategy mostly using affinity-purification and MS-based protein identification methodology has changed dramatically understanding the post-transcriptional assembly of ribosome in the past half decade, but mostly applied to the analysis for yeast cells (4). On the contrary to the great advancement in yeast, however, only small volumes of works have been done for not only human but also other mammals (5). The major obstacle to study ribosome biogenesis in mammals, especially human, is difficulty in doing genetic analysis and obtaining precursors of ribosome with an enough amount and with high purity. To overcome those problems, informatics approach has tried currently to assign function to uncharacterized proteins in about 700 nucleolar proteins by data integration coupled to a machine-learning method, and proposed a draft of the human ribosome biogenesis pathway encompassing 74 proteins, of which 49 were clamed to be previously uncharacterized proteins (5). This kind of approach is certainly useful to make working hypothesis; however, it cannot be proved without any experimental evidence. The recent advances of proteomic technologies can now overcome many of the difficulties associated with the analysis of human and/or mammalian ribosome biogenesis and provide proving experimental evidence for the predictions obtained from informatics point of views. In this presentation, we would like to talk about our continuing efforts for identifying trans-acting factors involved in various stages of ribosome biogenesis in human cells. We have used more than 30 proteins as affinity baits to isolate their associated protein complexes, and have so far assigned about 90 probable human trans-acting factors that are homologues to those of yeast involved in ribosome biogenesis, as well as about equal number of non-ribosomal proteins that have no known function in ribosome biogenesis. Focusing on the non-ribosomal proteins, we have found several novel trans-acting factors that are involved in human ribosome biogenesis but have no yeast counterparts. Based on these and the other results, we will discuss the characters of human pre-rRNP complexes as well as a possible outline of dynamic process of human ribosome biogenesis.

References

1. Murayama A. *et al*., (2008) *Cell*, **133**:627-639.
2. Martin DE. *et al*. (2004) *Cell,* **119**:969-979.
3. Wade JT. *et al*., (2004*) Nature* **432**:1054-1058.
4. Takahashi N. *et al*., (2003) *Mass Spectrom. Rev*., **22**:287 - 317.
5. Takahashi, N. and Isobe, T., in Proteomic Biology using LC/MS, John Wiley & Sons Inc., (2007) pp1-254.

Nobuhiro Takahashi

Professor

United Graduate School of Agriculture,
Tokyo University of Agriculture & Technology, Japan

ntakahas@cc.tuat.ac.jp

TEL: +81-42-367-5709 FAX: +81-42-367-5709

Education

Tokyo Metropolitan University, Ph.D., 1980, Biochemistry

Positions & Employment

1981-1983 Research Associate (Visiting Biologist)
1983-1987 Assistant Research Scientist (assistant research professor) with distinguished professor Frank W. Putnam, Department of Biology, Indiana University (Bloomington, Indiana, U.S.A.),
1987-1997 Chief Scientist, Corporate Research & Development Laboratory, Tonen Corporation (Saitama, Japan)
1997- present Professor, Department of Agriculture (Applied Biological Science)
Graduate School of Agriculture (Molecular Biology)
United Graduate School of Agriculture, Tokyo University of Agriculture & Technology (Tokyo, Japan)

Membership

Steering Committee for Petroleum Bioprocess (Petroleum Energy Center)
International Science Cooperation Committee (Japan Society for the Promotion of Science)
Science & Technology Expert Committee of Science Council (Ministry of Education, Culture, Sports, Science, and Technology of Japan)
Japanese Industrial Standard Committee (Ministry of Economy, Trade and Industry of Japan)

L-13

Protein interaction specificity: using 3D structure to unveil compensatory effects in protein interaction networks

Patrick Aloy

Institut for Research in Biomedicine, Spain

Proteins are the main perpetrators of most cellular tasks. However, they seldom act alone and most biological processes are carried out by macromolecular assemblies and regulated through a complex network of protein-protein interactions. Thus, modern molecular and cell biology no longer focus on single macromolecules but now look into complexes, pathways or even entire organisms. The many genome-sequencing initiatives have provided a near complete list of the components present in an organism, and post-genomic projects have aimed to catalogue the relationships between them. The emerging field of systems biology is now mainly centered on unraveling these relationships. However, all these interaction maps lack in molecular details: they tell us who interacts with whom, but not how. A full understanding of how molecules interact can be attained only from high resolution three-dimensional (3D) structures, since these provide crucial atomic details about binding. These details allow a more rational design of experiments to disrupt an interaction and therefore to perturb any system in which the interaction is involved. In this talk, I will discuss how the use of protein sequences and high-resolution 3D structures can help to reveal the molecular bases of how macromolecular complexes and cell networks operate.

Patrick Aloy

ICREA Research Professor

Institut for Research in Biomedicine, Spain

patrick.aloy@irbbarcelona.org

TEL: +34-93-4039690 FAX: +34-93-4039954

Dr. Patrick Aloy is an ICREA Research Professor and Principal Investigator of the Structural Systems Biology lab at the IRB. He obtained his BSc in Biochemistry and a MSc in Biotechnology by the Autonomous University of Barcelona, Spain, and spent six years as postdoctoral researcher and staff scientist at the European Molecular Biology Laboratory, Heidelberg, Germany. For eleven years, Dr. Aloy has been developing and implementing new technologies and algorithms, applying state-of-the-art methods to specific problems and bridging the gap between theoretical models and experiments in different disciplines. In the lasts years, he has pioneered system-scale analyses of macromolecular assemblies and networks using high-resolution three-dimensional structures, which has become a new discipline in structure prediction. Dr. Aloy has over 40 publications in first-rate journals, with over 1300 citations and remarkable press coverage, illustrating the scientific and social relevance of the work.

L-14

Innovative mass spectrometry technology for the study of cell signaling

Donald F. Hunt

Department of Chemistry, University of Virginia, USA

This lecture will focus on several different aspects cell signaling and the mass spectrometry technologies developed to study them. By using electron transfer dissociation (ETD)-tandem mass spectrometry it is now possible to analyze intact proteins on a chromatographic times scale (1 protein/2-5 sec). Proteins are converted to gas-phase, positive ions by electrospray ionization and then allowed to react with fluoranthene radical anions. Electron transfer to the multiply charged protein promotes random fragmentation of amide bonds along the protein backbone. Multiply charged fragment ions are then de-protonated in a second ion/ion reaction with the carboxylate anion of benzoic acid. The m/z values for the resulting singly, doubly, and triply charged ions are used to read a sequence of 15-60 amino acids at both the N and C termini of the protein. This information, along with the measured mass of the intact protein, is used to identify unknown proteins, to confirm the amino acid sequence of a known protein, to detect post-translational modifications, and to determine the presence of possible splice variants. Applications of this technology to the study of class I and class II antigen processing pathways, labile post-translational modifications (phosphorylation and O-GlcNAcylation), and complex patterns of post-translational modifications on histone proteins that regulate gene expression, stem cell differentiation, reprogramming of DNA in the egg, and changes of phenotype that do not involve altering the sequence of DNA (epigenics) will be discussed.

Donald F. Hunt

Professor

Departments of Chemistry and Pathology,
University of Virginia, USA

dfh@virginia.edu

TEL: +1-434-924-3610 FAX: +1-434-924-3567

Prof. Donald F. Hunt joined the faculty at the University of Virginia as an assistant professor in September, 1968 and was promoted to associate professor and full professor in 1973 and 1978, respectively. In 1993 he was promoted to the rank of University Professor with appointments in both Chemistry and Pathology. Prior to assuming these positions, he spent a year at the Massachusetts Institute of Technology as a National Institute of Health Postdoctoral Trainee in Mass Spectrometry under the guidance of Professor Klaus Biemann. The principal investigator obtained both his B.S. and Ph. D. (1967) degrees from the University of Massachusetts. Research for the doctoral dissertation was carried out under the direction of Professors Marvin Rausch and Peter Lillya in the area of organotransition metal chemistry. Prof. Hunt was chosen as a recipient of both an NIH Fogarty Senior International Fellowship and a John Simon Guggenheim Fellowship in 1981-82. In 1990, he received the Charles H. Stone Award sponsored by the American Chemical Society. In 1992 he was named Virginia's Outstanding Scientist and also received the Pehr Edman Award for outstanding achievements in the application of mass spectrometry to the contemporary microsequence analysis of proteins. The Distinguished Contribution Award from the American Society for Mass Spectrometry was presented to Dr. Hunt in 1994 for his development of electron capture negative ion mass spectrometry. In 1996 he was the first recipient of the Christian B. Anfinsen Award from the Protein Society for development of new technology in the field of protein chemistry. He received the Chemical Instrumentation Award sponsored by the American Chemical Society in 1997. This award recognizes Prof. Hunt for development of instrumentation capable of sequencing peptides and proteins at the attomole level. In 2000, Prof. Hunt was the recipient of both the Frank F. Field and Joe L. Franklin award presented by the American Chemical Society for outstanding achievement in the field of mass spectrometry and the Thomson Medal from the International Mass Spectrometry Society. In 2006 and 2007, Prof. Hunt was the recipient of Distinguished Accomplishment Awards from the Human Proteome Organization (HUPO) and ABRF, respectively. He is a co-inventor on more than 25 patents and patent applications and has over 350 scholarly publications to his credit. Professor Hunt is a consultant to Thermo Scientific Corp.

L-15

Novel MS-based proteomics technologies for dissection of post-translational modification networks

Yingming Zhao

Department of Biochemistry, UT Southwestern Medical Center, USA

More than 200 known protein post-translational modifications (PTMs) have been reported. However, only a small portion of them have been extensively characterized. Chemical nature of the protein modifications offers very limited insights into their roles in biochemical pathways. The vast number of uncharacterized protein modifications suggests that new technologies remain to be developed for their functional studies. In this presentation, we will highlight several technologies, which we developed in the fast few years, to address various issues on molecular characterization of PTMs, including global profiling and quantification, identification and validation of novel PTMs, studies of PTM cross-talks in the same proteins, identification of PTM-specific binding domains, and regulations of PTM modification enzymes. These methods integrate sensitive mass spectrometry and efficient isolation of proteins/peptides of interest, therefore vastly increasing the quality of data generated from such targeted proteomics methods. These methods provide powerful tools for the studies of PTM networks and their roles in cellular physiology.

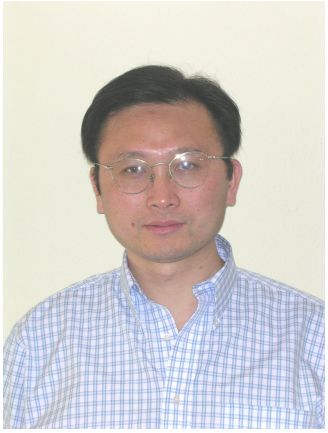
Yingming Zhao

Associate Professor

Department of Biochemistry,
UT Southwestern Medical Center, USA

yzhao@biochem.swmed.edu

TEL: +1-214-648-7947 FAX: +1-214-648-2797



Dr. Yingming Zhao got his Ph.D. in 1997. from The Rockefeller University under the supervision of Professor Brian T Chait. He has been as Assistant Professor and Associate Professor in the department of Biochemistry at UT Southwestern Medical Center from 2000. He is going to join Ben May Department of Cancer Research at The University of Chicago after this meeting. His research focuses on developing new mass spectrometry-based proteomics technologies for systematic analysis of protein modifications and applying them to addressing important biological problems.

L-16

Linking protein arrays to signalling pathways and diseases

Jan van Oostrum

Zeptosens - a Division of Bayer (Schweiz) AG, Switzerland

Molecular signalling pathways are frequently triggered by extra-cellular molecules binding receptors and activating relay systems inside cells, leading to processes that affect cellular behaviour and fate. For many genetic disorders a link between disease and signalling pathways have been established and consequently a systematic analysis of dynamic cellular networks provides an opportunity for pharmaceutical discovery, by taking into consideration the complex biological context of drug targets, rather than observing the targets in isolation. Such analyses are, perhaps, ideally suited for a systems biology approach that integrates experimental data with computational modelling with the aims of discovering and validating new drug targets and biomarkers, as well as predicting potential off target・effects of drug candidates. A proteomics platform based on reverse・protein arrays (RPA) is particularly suitable to monitoring cell-signalling events. These arrays are based on the principle that complex protein mixtures or proteomes (such as cell or tissue lysates) are spotted in an array format and probed with selected fluorescent antibodies in a multiplexed manner. To ensure high levels of sensitivity and signal to noise ratio of these RPAs, we are using planar waveguide technology. The advantage of the evanescent field fluorescence detection ensures that only analyte-bound fluorescent antibodies contribute to the signal. Due to the high sensitivity and high throughput capability of the reverse protein array approach it will be feasible to obtain protein expression profiles and signalling pathway information on a wide variety of cell lines and tissue samples. We will address selected applications including the elucidation of the dynamic aspects of pathway events and the profiling of compounds to reveal signalling and cross-pathway effects of drug candidates. In addition analysis of healthy versus disease tissue (including animal models) will provide insights into the pathways・underlying pathologies and provide a platform for molecular diagnostics.

Jan van Oostrum

Head Business Development

Zeptosens - a Division of Bayer (Schweiz) AG, Switzerland

jan.van_oostrum@zeptosens.com

TEL: +41-61-726-8187 FAX: +41-61-726-8170

Dr. Jan van Oostrum was recently appointed as Head Business Development at Zeptosens and is the former Head of the Protein Science and Technology Unit at the Novartis Institutes for Biomedical Research in Basel, Switzerland. Dr. Jan van Oostrum received his Ph.D. from Columbia University in New York and holds an affiliate faculty appointment at the Department of Anatomy and Cell Biology at the McGill University in Montreal.

His main interest is on integration and application of protein micro-array technologies in order to obtain a "systems" perspective on diseases linked to molecular signaling pathways.

L-17

Mechanical modulation of ATP-binding and hydrolysis by single F1-ATPase molecule

Hiroyuki Noji

Institute of Scientific and Industrial Research, Osaka University, Japan

F1-ATPase is a molecular motor protein that rotates the gamma rotor subunit unidirectionally against the stator ring driven by ATP hydrolysis. In comparison to other molecular motors, the prominent feature of F1-ATPase is the high energy conversion efficiency from chemical energy into mechanical work. To realize its mechanochemical coupling mechanism, we determined the rate and equilibrium constants of ATP binding and ATP hydrolysis as the functions of the rotary angle of the gamma subunit by single molecule manipulation. ATP-binding and hydrolysis states were shown to be progressively stabilized with the gamma rotation, which implied that in F1, the energy derived from each catalytic step is gradually converted into mechanical rotation to avoid energy dissipation. It was also revealed that the equilibrium of ATP binding exponentially increased with the rotation, while that of hydrolysis increased slightly. This proves that F1 generates a large torque during the progressive ATP-binding process, but not so much during the hydrolysis step.

Hiroyuki Noji

Professor

Institute of Scientific and Industrial Research (ISIR),
Osaka University, Japan

hnoji@sanken.osaka-u.ac.jp

TEL: +81-6-6879-8481 FAX: +81-6-6875-5724

Education

1993	BA, the Department of Bioscience of the Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology.
1995	Master, the Department of Bioscience of the Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology.
1997	PhD, the Department of Electronic Chemistry, Interdisciplinary Graduate School of Science and Engineering, Tokyo Institute of Technology.

Positions & Employment

1998-2000	Post doctoral researcher, CREST team 13, JST
2000-2001	Researcher, PRESTO, JST
2001-2005	Associate Professor, Institute of Industrial Science, The University of Tokyo
2005-prenset	Professor, Institute of Scientific and Industrial Research, Osaka University

Honors

2006	JSPS Prize, Japan Society for the Promotion of Science
1998	Grand Prize, Amersham Pharmacia Biotech & Science Prize for Young Scientists
1999	Tejima Prize for Doctoral Dissertation Award, Tejima Seiichi Commemorative Foundation

L-18

Genetic code reprogramming and RaPID system

Hiroaki Suga

Research Center for Advanced Science and Technology, The University of Tokyo,
Department of Chemistry and Biotechnology, Graduate School of Engineering, The University of Tokyo,
Department of Advanced Interdisciplinary Studies, Graduate School of Engineering, The University of Tokyo, Japan

Genetic code reprogramming is a new emerging methodology that enables us to synthesize non-standard peptides containing multiple non-proteinogenic amino acids using translation machinery. In this lecture, I shall discuss the method development of the genetic code reprogramming using flexizyme system that facilitates the charging process of a variety of non-proteinogenic amino acids onto tRNAs bearing designated anticodons. I shall also present some of the recent demonstrations of the synthesis of non-standard peptides with cyclic structure or/and altered backbones employing this technology, and future applications to peptide drug discoveries.

Hiroaki Suga

Professor

Research Center for Advanced Science and Technology,
The University of Tokyo, Japan

hsuga@rcast.u-tokyo.ac.jp

TEL: +81-3-5452-5495 FAX: +81-3-5452-5495

Education

Massachusetts Institute of Technology, Cambridge, MA Ph.D. 1994

Positions & Employment

1994-1997	Post-doctoral Fellow, Harvard Medical School/Massachusetts General Hospital, Boston, MA.
1997-2002	Assistant Professor, Dep. of Chemistry, University at Buffalo (SUNY)
2002-2003	Associate Professor, Dep. Of Chemistry, University at Buffalo (SUNY)
2003-2005	Associate Professor, Research Center for Advanced Science and Technology, University of Tokyo
2005-present	Professor, Research Center for Advanced Science and Technology, University of Tokyo

L-19

Proteins in action: Monitored by tr(time-resolved) FTIR spectroscopy

Klaus Gerwert

Chair of Biopyhsics, Faculty of Biology and Biotechnology, Ruhr University Bochum, Germany

In the Postgenom era proteins are coming into the focus in the life sciences. Proteins are the nanomachines that perform the work in living organisms or are the receptors and mediators for external signals. By NMR and x-ray the three dimensional structural architecture of proteins are determined. In order to elucidate the function, time-resolved methods have to be applied. FTIR difference spectroscopy can be used to monitor the reactions within proteins at the atomic level with ns time-resolution up to days [1]. In combination with site directed mutagenesis or isotopically labelling the IR bands can be clear cut assigned to specific amino acids or ligands. This provides in combination with structural models also spatial resolution. Based on fast scan studies on bacteriorhodopsin the key catalytic residues, asp 85 and asp 96 and their protonation kinetics are identified and summarized in a first detailed proton pump model [2]. Their structural arrangement as resolved in succeeding X-ray experiments by several groups supports this proposal. The X-ray structural model at 1.55 Å resolves in addition the oxygens of internal water molecules. Based on succeeding step scan FTIR measurements the interplay between these water molecules, a strongly hydrogen bonded water, a dangling water and a protonated water complex is elucidated in detail. It results in a controlled Grothuis proton transfer from the central proton binding site to the protein surface. [3,4]. A similar mechanism might apply in the photosynthetic reaction center [5] and the cytochrome oxydase [6]. The step scan approach is also successfully applied to the photoactive yellow protein [7]. The difference technique requires fast triggering of the protein reaction, which is easy to accomplish for the chromoproteins as described before. Progress for the investigation of non chromophoric proteins is acquired by developing a micro mixing cell for FTIR studies, allowing mixing times in the sub ms time range. This cell is used to investigate protein folding reactions [8]. Alternatively, photolabile caged compounds can be applied. Using caged GTP the GTPase mechanism of the protooncogen Ras is investigated [9,10]. Also its protein-protein interaction with the GAP protein could be studied time-resolved [11,12]. This provides a detailed insight into the catalytic mechanism by which GAP activates the GTPase by five orders of magnitude. The activation by GAP proteins is a central process in the signal transduction. In oncogenic Ras this activation process is inhibited and involved in uncontrolled cell growth. The study proves that the approach can be extended to protein-protein interactions. Recently, beside rection within the active site of a protein, also the surface change of a protein leading to protein-protein interactions is monitored [13].

References

1. Koetting, C., Gerwert, K., *Chem Phys. Chem* **6**, 881-888 (2005)
2. Gerwert, K., Souvignier, G. and Hess, B., *Proc. Natl. Acad. Sci. USA* **87**, 9774-9778, (1990)
3. Garczarek, F., Brown, L.S., Lanyi, J.K., Gerwert, K., *Proc. Natl. Acad. Sci USA* **102**, 3633-3638 (2005)
4. Garczarek, F., Gerwert, K. *Nature* **439**, 109-112 (2006)
5. Remy, A., Gerwert, K., *Nature Struct. Biol.* **10**, 637-644 (2003)
6. Lueben, M., Gerwert, K., *FEBS Letters* **397**, 303-307 (1996)
7. Brudler, R., Rammelsberg, R., Whoo, T. T., Getzoff, E. D., Gerwert, K., *Nature Struct. Biol.*, **8**, 265 (2001)
8. Kauffmann, E., Darnton, N. C., Austin, R. H., Gerwert, K., *Proc. Natl. Acad. Sci., USA* **98**, 6646 (2001)
9. Cepus,V., Goody, R.S., Gerwert, K., *Biochemistry* 1998, **37**, 10263-10271, (1998)
10. Allin, C., Gerwert, K., *Biochemistry*, **40**, 3037-3046, (2001)
11. Allin, C., Ahmadian, M. R., Wittinghofer, A., Gerwert, K., *Proc. Natl. Acad. Sci., USA* **98**, 7754 (2001)
12. Koetting, C., Blessenohl, M., Suveyzdis, Y., Goody, R.S., Wittinghofer, A., Gerwert, K. *Proc. Natl. Acad. Sci. USA* **103**, 13911-13916 (2006)
13. Koettting, C., Kallenbach, A., Suveyzdis, Y., Eichholz, C., Gerwert, K., *ChemBioChem*, **8**, 781-787 (2007)

Klaus Gerwert

Professor

Chair of Biopyhsics, Faculty of Biology and Biotechnology,
Ruhr University Bochum, Germany

gerwert@bph.rub.de

TEL: +49-234-32-24461 FAX: +49-234-32-14238

Education

1975-1981	Study of Physics at Muenster University ("very good; 1.0")
1982-1986	Ph. D. study of Biophysical Chemistry at University Freiburg
1986	Dr. rer. nat., University of Freiburg, Faculty of Chemistry and Pharmacy ("summa cum laude")

Positions and Employment

1986-1990	research scientist at the Max-Planck-Institute in Dortmund (Prof. Benno Hess)
1990	"Visiting investigator" at Scripps Research Institute, La Jolla, USA (Prof. Peter Wright)
1990-1993	Heisenberg-fellow of the Deutsche Forschungsgemeinschaft at MPI Dortmund and Scripps Research Institute
Since 1993	Chair of Biophysics, Faculty of Biology

Honours

1990	Heisenberg fellowship of the Deutsche Forschungsgemeinschaft
1991	"Karl Arnold Jansen" Award of the "Rheinisch-Westfälische Akademie der Wissenschaften", Düsseldorf (personal prize, awarded by 20.000 DM)
1997	Award "Leuchtturmprojekt molekulare Biophysik" of NRW
2006	Award „Innovationspreis Ruhr 2006" by Ministerpresident NRW (Dr. J. Rüttgers and Krupp Foundation) (personal prize awarded by 25.000 Euro)

L-20

Proteomics identification of proteins involved in colorectal cancer metastasis

Xuxiao Zhang[1], Yizhen Ng[1], Hwee Tong Tan[2], Sandra Tan[2], Shing Chuan Hooi[3],
Maxey C.M. Chung[1,2]

[1]Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore,
[2]Department of Biological Sciences, Faculty of Science, National University of Singapore,
[3]Department of Physiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

Colorectal cancer (CRC) is the third most common malignancy and the second leading cause of cancer deaths in developed countries. About 30% - 50% of all patients with CRC die within 5 years after diagnosis. The high mortality of CRC is largely due to extensive metastatic spread of the disease, with secondary hepatic tumours being the most common occurrence. Acquisition of metastatic phenotype by cancer cells thus represents one of the most lethal aspects of cancer progression. Patient survival in CRC will greatly benefit from earlier recognition (and intervention) of metastasis but as yet, proper markers indicating metastases are not available.

E1 is a highly metastatic cell line that had been derived from hepatic metastases following splenic injection of HCT-116 primary colon cancer cell line into nude mice. Since E1 was derived from HCT-116, they share the same genetic background. Thus any proteome differences would most likely be due to the acquisition of metastatic potential of E1. To identify proteins associated with colorectal metastasis, we carried out comparative proteomics analysis of HCT-116 and E1 cells. Whole cell lysates and fractions obtained from an enrichment step of heparin affinity chromatography were analyzed by two dimensional difference gel electrophoresis (2-D DIGE) coupled with tandem mass spectrometry (MS/MS). Using whole cell DIGE, only 15 differentially expressed spots between HCT-116 and E1 were identified. However, heparin affinity pre-fractionation greatly increased the number of differentially regulated protein spots detected. A total of 30 and 98 differentially regulated spots were detected in the unbound and bound DIGE fractions respectively. In total, 143 protein spots were detected to be differentially expressed between the 2 cell lines and they corresponded to isoforms of 86 different proteins as identified by MS analyses. A majority of these proteins are implicated in cell growth, survival, motility, invasiveness and angiogenesis, which are indispensable steps in the metastatic cascade. Using these approaches, our work has thus identified a set of proteins that may play significant roles in the metastatic progression of colorectal cancer. They may represent candidate biomarkers for colorectal cancer metastasis as well as potential therapeutic targets for treatment of metastatic colorectal cancer.

We have also proceeded to functionally validate one of the proteins, stathmin, with the view to establish its precise role in the metastasis cascade. In addition to this being the first report of stathmin upregulation in colorectal cancer metastasis, it was also the most upregulated protein in the E1 cell line as compared to its parental HCT116 cell line. RNA interference was carried out to investigate the functional effects of stathmin knockdown in E1. Our results showed that knockdown of stathmin in E1 did not affect cell viability but decreased cell migration significantly. In addition, stathmin expression levels were also shown to correlate strongly with increasing metastatic potential in other paradigms/models of colorectal metastasis that we examined. In conclusion, our findings suggest that stathmin is a potential biomarker and therapeutic target for treatment of colorectal cancer metastasis.

Maxey C.M. Chung

Associate Professor

Departments of Biochemistry and Biological Sciences,
Yong Loo Lin School of Medicine and Faculty of Science,
National University of Singapore, Singapore

bchcm@nus.edu.sg

TEL: +65-6516-3252 FAX: +65-6779-1453

Dr. Maxey Chung (Ph.D., Victoria University of Wellington, New Zealand) holds joint appointments as Associate Professor at the Departments of Biochemistry, Yong Loo Lin School of Medicine and Biological Sciences, Faculty of Science at the National University of Singapore. He is also currently the Principal Investigator of the Oncoproteomics Laboratory in DBS. His main research interest is in the field of cancer biomarker discovery, especially for gastrointestinal cancers. In recent years, his laboratory has also focused on the identification and elucidation of the proteins and pathways involved in cancer metastasis as well as cancer cell response to HDACi (histone deacetylase inhibitor) treatment such as butyrate using functional proteomics approaches.

He is currently the Secretary General of AOHUPO (Asian Oceanian Human Proteome Organization) as well as an elected council member of HUPO (Human Proteome Organization). In addition, he is a Senior Editor of Proteomics, Proteomics - Clinical Applications and Proteomics - Practical Proteomics, and is also a regular reviewer for several leading biochemical and proteomics journals.

L-21

Cancer proteomics for personalized medicine

Tadashi Kondo

Proteome Bioinformatics Project, National Cancer Center Research Institute, Japan

Cancer is a diverse disease, and the present clinical and pathological diagnostic modalities have obvious limitation in the prediction of clinical outcome. The next level of predictive molecular diagnostics using novel biomarkers are expected to best-optimize the existing therapeutic strategy. We examined proteome contents in more than 1,000 tumor tissues using our original large format two-dimensional difference gel electrophoresis (2D-DIGE) system (1). By integrating 2D-DIGE data with clinico-pathological parameters, we concluded that proteome reflects the major malignant phenotypes of cancer and proteomics has a great potential to identify biomarker candidate proteins. For instance, 2D-DIGE data included key proteins corresponding to the response to treatment in lung adenocarcinoma (2), osteosarcoma and Ewing sarcoma, the early recurrence in liver cancer (3), and the metastasis post surgery in gastrointestinal stromal tumor (4). For certain proteins, the predictive performance was successfully validated in more than 100 cases by immunohistochemistry. Such proteins should be strong candidates for biomarkers in personalized medicine. The clinical application of these research results is our next challenge. To facilitate the integrative and comprehensive omics study, we take a part of Genome Medicine Database of Japan (GeMDBJ) (5). All proteome data by 2D-DIGE, protein annotations and clinico-pathologidal data are currently being integrated into this database. Proteome data of clinical samples in GeMDBJ will be opened in the next one year.

References

1. Tadashi Kondo and Setsuo Hirohashi. Application of highly sensitive fluorescent dyes (CyDye DIGE Fluor saturationdyes) to laser microdissection and two-dimensional difference gel electrophoresis (2D-DIGE) for cancer proteomics. *Nat Protoc.* **1**, 2940-2956, 2006.
2. Okano T and Kondo T et al. Proteomic signature corresponding to the response to gefitinib (Iressa, ZD1839), an epidermal growth factor receptor tyrosine kinase inhibitor in lung adenocarcinoma. *Clin. Cancer Res.* **13**, 799-805, 2007.
3. Yokoo H and Kondo T et al, Protein expression associated with early intrahepatic recurrence of hepatocellular carcinoma after curative surgery. *Cancer Sci.* **98**, 665-673, 2007.
4. Suehara Y and Kondo T et al. Pfetin as a prognostic biomarker of gastrointestinal stromal tumors revealed by proteomics. *Clin. Cancer Res.* **14**, 1707-1717, 2008.
5. https://gemdbj.nibio.go.jp/dgdb/DigeTop.do

Tadashi Kondo

Project leader

Proteome Bioinformatics Project,
National Cancer Center Research Institute, Japan

takondo@ncc.go.jp

TEL: +81-3-3542-2511(3004) FAX: +81-3-3547-5298

Dr. Tadashi Kondo is currently a Project Leader at Proteome Bioinformatics Project, the National Cancer Center Research Institute. Dr. Kondo graduated from Okayama University Medical School, and received M.D. and Ph.D. degree. He started cancer proteomics using 2D-PAGE in his Ph.D. course, and had postdoctoral training in the University of Michigan. Dr. Kondo has a major interest in application of cancer proteomics to biomarker development. He has established the largest gel-based proteomics laboratory in Japan, identifying proteins associated with important clinico-pathological features by collaborating with many clinicians and pathologists. He takes a proteomics part of Genome Medicine Database of Japan (GeMDBJ).

IAPSAP/MPSA

Pehr Edman Awards

Young Investigator Award

IAPSAP/MPSA 2008 Pehr Edman Awards

The Pehr Edman Award is given to individuals whose efforts have significantly advanced the fields of protein chemistry, protein structure analysis, or proteomics. The award honors and commemorates the work of Pehr Edman, the Swedish chemist principally responsible for developing the chemistry for sequencing proteins by removing amino acids from the amino terminus one at a time. The 2008 awards, which are supported by Applied Biosystems and The Waters Corporation, will be presented at MPSA2008 to:

Dr. Richard Perham
Department of Biochemistry, University of Cambridge, for his pioneering contributions to the structural analysis of proteins, protein complexes and filamentous viruses that have provided important insights for their assembly and function and for the development of techniques for protein modification.

Dr. Ettore Appella
Laboratory of Cell Biology, National Cancer Institute, NIH, for his work on deciphering the code through which posttranslational modifications modulate the activity of the p53 tumor suppressor and his contributions to characterizing the interactions of the T-cell receptor (TCR) with class I molecules of the major histocompatibility complex (MHC).

IAPSAP/MPSA 2008 Young Investigator Award

IAPSAP created the Young Investigator Award in 2000 to recognize promising young investigators who are beginning to advance the fields of protein chemistry, protein structure analysis, or proteomics. The 2008 IAPSAP/MPSA Young Investigator Award is supported by Agilent, Inc., and will be presented to:

Dr. Andreas Ladurner
European Molecular Biology Laboratory, Heidelberg, Germany, for his pioneering efforts to identify protein modules that recognize post-translational modifications in histone proteins and his protein engineering analysis of key components in the RNAi pathway.

Richard Perham

Professor
Department of Biochemistry
University of Cambridge
rnp1@cam.ac.uk
TEL: 44 1223 363710 FAX: 44 1223 338707

Dr. Richard Perham received his Ph.D. at the MRC Laboratory of Molecular Biology in Cambridge, in the laboratory of Drs. F. Sanger and J. I. Harris. He was a Helen Hay Whitney Fellow in Molecular Biophysics at Yale University with Professor F. Richards, before returning to Cambridge. He has been an EMBO Fellow in the Max-Planck-Institut fur Medizinische Forschung in Heidelberg, a visiting Professor at the University of New South Wales in Sydney, Australia, and a Fogarty International Scholar at the National Institutes of Health, Bethesda, Maryland, USA. Dr. Perham began his work at Cambridge on structural and mechanistic studies of glyceraldehyde 3-phosphate dehydrogenase. He and Ieuan Harris held the world record in the mid-1960s for determining the longest amino acid sequence (over 330 residues) of a protein, and they elucidated much of the mechanism associated with the thioester intermediate. In the late 1960s he uncovered the importance of charge-charge interaction between protein subunits in the self-assembly of tobacco mosaic virus capsids and later elucidated the novel mechanism of protein-DNA charge interaction that governs the assembly of filamentous bacteriophage virions. He introduced a number of important techniques in chemical modification of proteins, in particular based on reversible amidination and trifluoroacetylation of lysine residues. After some 30 years of effort, he and his colleagues have recently produced the first essentially complete description of the structure and assembly pathway of a multienzyme complex, the pyruvate dehydrogenase complex (molecular mass 10 MDa). In studying its dihydrolipoamide dehydrogenase component, he and his group were the first to demonstrate how to change the coenzyme specificity (NAD/NADP) and the kinetic mechanisms (ordered and allosteric) of an enzyme by rational mutagenesis and uncovered a new mechanism of active site cooperativity, distinct from allostery, that operates through a buried proton wire in the thiamine-dependent pyruvate decarboxylase. In parallel studies, he and his colleagues have demonstrated how to use filamentous bacteriophage capsids and the icosahedral cores of pyruvate dehydrogenase complexes as molecular scaffolds for the multiple display of foreign peptides and proteins, which has led to important insights on antigen recognition and the immune response in vaccine design.

Ettore Appella

Head
Chemical Immunology Section
Laboratory of Cell Biology
National Cancer Institute
National Institutes of Health
appellae@mail.nih.gov
TEL: 1-301-402-4177 FAX: 1-301-496-7220

Dr. Ettore Appella received his M.D. from the University Medical School, in Rome, Italy and then became a Research associate at Johns Hopkins University. After a post-doctoral fellowship at the Centre de Recherches sur les Macromolecules in Strasbourg, France, he began his research at the National Institutes of Health, in 1963. In 1979, he and his colleagues identified the p53 tumor suppressor protein, and they have continued p53 studies since that time. For more than 15 years they have contributed to deciphering the code through which posttranslational modifications to p53, including phosphorylations, acetylations, and methylations, modulate p53 activity and stability in response to cellular stresses including DNA damage induced by ionizing radiation, UV light, or chemical agents used in cancer chemotherapy. His recent work includes analysis of the functional effects of single or multiple knock-in mutations at sites of posttranslational modifications, especially in those tissues that show increased tumor development. In 1997, his group discovered a wild-type p53-induced phosphatase, Wip1 (PP2Cdelta or PPM1D), which functions as a negative regulator of the p38 MAP kinase- and ATM-p53 signaling pathways, essential for p53 activation. Another of his long time interests has been the interaction between CD8+ T cells and their cellular targets. His group has focused on how the T cell receptor (TCR) of CTL interacts with class I molecules of the Major Histocompatibility Complex (MHC). Using a model system they have developed to examine cross-recognition of different MHC/peptide complexes by a single TCR, they have been probing the structural, functional, and biochemical features of ligand engagement.

Andreas Ladurner

Group Leader
EMBL Heidelberg
ladurner@embl.de
TEL: 49-622-1387-8156 FAX: 4962-2138-7518

Dr. Ladurner earned his Ph.D. in chemistry from the University of Cambridge in 1998. He was awarded a Wellcome Trust International Fellowship at the University of California, Berkeley, and then worked there as a Research Associate. After serving as an editor for the Nature Publishing Group in New York, he returned to Europe in 2003 to take up his current position as Group Leader in the European Molecular Biology Laboratory in Heidelberg.

Dr. Ladurner has pioneered the identification of protein modules that recognize post-translational modifications in histone proteins. His long-term goal is to decipher how dynamic changes in nucleosome structure contribute to the regulation of gene expression. Using a variety of biophysical, structural and protein engineering approaches, his work identifies and characterizes protein modules and linear peptide motifs involved in the recognition of chromatin and in chromatin-targeted gene silencing mechanisms. In particular, he analyzes proteins that recognize specific post-translational modifications in histones, has identified a metabolite-binding function in a human histone, and also studies the mechanisms of histone exchange by so-called histone chaperones.

His protein engineering analysis of key components in the RNAi pathway have uncovered the existence of a conserved linear peptide motif which recognizes specific Argonaute proteins. These motifs, termed Ago hooks, mediate distinct biological functions. These range from chromatin silencing in fission yeast, specific forms of DNA transcription in plants and finally repression of protein translation by micro-RNAs in animals. The identification of novel regulatory interactions between proteins and ligands using computational, quantitative and structural approaches continues to reveal fundamentally novel paradigms of biological control in gene expression.

Short talks

S-01 ~ S-07

S-01

Secondary Structure Mimics of protein-protein interactions

Siddhartha Roy[1], Amlanjyoti Dhar[1], Shampa Mullick[1], Atanu Maiti[1], Santu Bandyopadhyay[1], Srijata Mukherjee[1], David Weber[2], Paul T. Wilder[2], Joseph Markowitz[2]

[1]*Division of Structural Biology and Bioinformatics, Indian Institute of Chemical Biology, 4, Raja S.C. Mullick Road, Kolkata 700 032, India,* [2]*Department of Biochemistry and Molecular Biology, University of Maryland School of Medicine, 108 N. Greene Street, Baltimore, Maryland 21201, USA.*

Protein-protein interactions form a major part of the signal transduction network in cells. Specific inhibition of protein-protein interaction can be an effective tool of chemical genetics as well as future therapeutic applications. S100B is a protein which is over-expressed in melanomas and other cancers. It deactivates p53 network by down regulating p53 levels. Mdm2 is a well-known down-regulator of p53 levels and is over-expressed in many cancers. Design, synthesis and use of α-helical analogs against both these targets will be presented. Implications for p53 network will be discussed.

S-02 (P-029)

Gene annotation in *Toxoplasma gondii*

Ruth Hogue Angeletti, Joseph Dybas, Carlos Madrid, Dmitrij Rykunov, Edward Nieves, Fa-Yun Che, Hui Xiao, Kami Kim, Louis Weiss, Andras Fiser

Biodefense Proteomics Research Center, Albert Einstein College of Medicine, USA

Toxoplasma gondii, a member of the phylum Apicomplexa, can be fatal to a fetus or to immunocompromised hosts. Its ability to be transmitted by water makes it a potential agent for bioterrorism. Over the past three years, four gene prediction data sets have been published for this organism, for which ca. 7000 genes are expected. The official, final version ("Release-4") genome annotation released for *T. gondii* in 2006, updated the previous three annotations (TIGRSCAN, TWINSCAN, GLIMMER) and the available sequence data already deposited in the GenBank. However, results of high throughput mass spectrometry experiments revealed that none of the gene predictions is satisfactory alone, including Release-4. A non-overlapping subset of these previous predictions is compiled by all-to-all sequence comparisons and resulted in 25236 *T. gondii* proteins. Mass spectrometry experiments on cytoskeletal and membrane fractions were carried out by LC-MS/MS, and searched with MASCOT. Gene predictions were verified by cross referencing predicted sequences with mass spectrometry peptide hits. A total of 8372 predicted proteins were identified by mass spectrometry in *T. gondii* to date. These predicted proteins cluster into 4160 groups of overlapping predictions and they were identified by 14455 mass spectrometry peptide hits that gave an average 24.32% sequence coverage. Out of these 4160 clusters of validated protein predictions only 2101 contain Release-4 sequences (51%). However 105 sequences out of these are not validated by any direct mass spec peptide hit, just a related, partially overlapping sequence in the same cluster that was obtained from other gene prediction sources. This means that only 2022 Release-4 proteins could be directly validate suggesting a mere 48.5% correct prediction rate in the official Release-4 genome annotation. The results underline the need for more accurate gene annotation tools. This study is one of the first whole genome proteomics validation efforts that could serve as a prototype to explore gene structures of strategic organisms. (Supported by NIH/NIAID contract HHSN266200400054C)

S-03

Liquid chromatography - mass spectrometry using ion trap based electron capture dissociation

Shinya Ito[1,2], Jared Bushey[1], **Takashi Baba[1,3]**, Gary L. Glish[1]

[1]University of North Carolina, Chapel Hill, North Carolina, USA [2]Hitachi High-Technologies, JAPAN [3]Hitachi, Ltd., Japan

Electron capture dissociation (ECD) tandem mass spectrometry (MS/MS) has become a powerful method to obtain peptide and protein sequence information. Generally ECD provides more sequence coverage than standard collision induced dissociation (CID), and ECD also allows posttranslational modifications to be retained by the peptide/protein product ions. ECD MS/MS has historically been performed in Fourier transform ion cyclotron resonance (FT-ICR) instruments. However FT-ICR mass analyzers are relatively slow; thus it is challenging to utilize them on-line with liquid chromatography (LC). A new approach to effecting ECD in a radio frequency (rf) ion trap, with subsequent product ion analysis using a time-of-flight (TOF) analyzer, offers a promising alternative to FT-ICR for performing ECD MS/MS on the LC time scale. LC ECD MS/MS results using this new approach for implementing ECD are reported here.

A linear rf ion trap with a magnet is an excellent choice for an ECD device. The linear rf ion trap is composed of a four rod set that establishes an ion trapping electrodynamic field. The parent ions are focused on the center line of the linear ion trap, where the electron beams is traveling, so the overlap between electrons and ions is automatically optimized. The linear electrodynamic and static magnetic fields reduce disturbance of both electron trajectory and electron energy, allowing efficient electron capture rates. These two features allowed fast ECD applicable to the LC time scale.

The versatile rf ion trap based ECD device allows several dissociation techniques to be used: ECD, Hot ECD, CID and any combination of them. Hot ECD, which uses electrons with kinetic energy higher than ECD, provides more efficient cleavage than ECD while labile moieties still remain intact. This is especially useful for LC measurements in the field of glyco-proteomics.

We applied the LC – rf ECD to tryptic fragments of bovine fetuin. Experiments were performed on a modified nano-Frontier LC/MS (Hitachi High Tech). The mass spectrometer is a tandem linear ion trap/TOF (LIT/TOF) with an rf-ECD cell added between the LIT and TOF. Bovine fetuin, (Sigma) was digested with trypsin. Sequential Hot ECD and CID MS/MS was applied to the digests. Eight types of N-glycopeptides and two types of O-glycopeptides were detected, which were identified as glycans, modification sites, or both.

S-04

Defining a new route to absolute protein quantitation

Mark McDowall[1], Jim Langridge[1], Hans Vissers[1], Therese McKenna[1], Iain Campuzano[1], Hans Aerts[2], Martha Stapels[3], Craig Dorschel[3], Scott Geromanos[3]

[1]*Waters Corporation, MS Technologies Center, Manchester, UK* [2]*AMC, University of Amsterdam, Department of Biochemistry, NL* [3]*Waters Corporation, Milford MA, USA*

During the past decade mass spectrometry has become widely accepted as an essential tool to better understand protein function, facilitating both the identification and quantification of proteins in complex samples. We, and others, have previously described a novel approach to mass spectrometry based protein identification [1-3] that facilitates the simultaneous acquisition of qualitative and quantitative information, in a data independent fashion without the use of isotope labelling.

We have used this approach to generate absolute quantification values for proteins contained in biological systems [4]. We have extended this to study samples from a range of organisms, as well as to construct protein abundance curves for specific tissues, cell lysates and biofluids. An important aspect of this absolute quantitation procedure is that it allows sample loading onto a given analytical column to be determined and optimized, to ensure that ideal chromatographic and mass spectrometric performance is obtained. This results in the maximum number of peptide and proteins being determined from the sample, whilst maintaining maximum accuracy for quantitative measurements. This we believe is an aspect often overlooked in modern proteomics experiments. Absolute quantification also provides a mechanism to define the protein stoichiometry present within a sample. In this manner protein pathways and families can be discerned and compared, and the mechanism by which proteins interact can be probed.

It is a common proteomics experiment to use relative quantitation, to determine information about protein expression changes within an experiment. In many respects this can be considered as an isolated island of information that can only be compared within a given experiment. The possibility of performing absolute quantitation of proteins generates a bridge between data sets, allowing the comparison of protein amounts across experiments, instruments, organisms, and laboratories.

In this presentation we will focus on the absolute quantification of proteins from a variety of different biological samples using label-free LC-MS. We will show absolute quantitation data from cell lysates of *E.coli*, and the monitoring of known Gaucher disease biomarkers from the plasma of patients undergoing therapy.

1 Bateman et al, *JASMS* 2002 Jul;**13**(7):792-803.
2 Purvine et al, *Proteomics.* 2003 Jun;**3**(6):847-50.
3 Silva et al, *Anal Chem.* 2005 Apr 1;**77**(7):2187-200.
4 Silva et al, *Mol Cell Proteomics.* 2006 Jan;**5**(1):144-56.

S-05

Targeted LC/MS/MS techniques characterize recombinant therapeutics, including heterogeneity, and low-frequency post translational modifications

Matthew M. Champion

Applied Biosystems, Proteomics and Small Molecule Division, USA

Accurate characterization of recombinant protein therapeutics is essential to defining the nature of the observed biological activities. In particular, describing the complete sequence of a developed protein including modifications and sequence changes is non-trivial. Here, we developed a series of tools to target and characterize a set of recombinant fusion-protein therapeutics, and monoclonal antibodies using a novel combination of mass spectrometric tools and bioinformatics. Differential gas-phase analyses were automatically collected to describe a large degree of the primary structure of these proteins including the novel peptides created across the fusion-protein junction. Additionally, these were combined with targeted Multiple-Reaction-Monitoring injections to detect and sequence alternative *in silico* hypotheses about the presence of post translational modifications of these proteins, including variable oxidation, carbamylation of Lys (K) residues, and N and C terminal heterogeneity and modification. Specifically, we were able to identify novel sites of modification, including acetylation and phosphorylation of recombinant therapeutics and utilized aspects of hybrid triple-quadrupole mass spectrometry to quantify these defects from the desired sequence. The non-targeted LC/MS/MS characterization was then combined with the targeted approaches, and the sequences are assembled using a novel algorithm capable of interrogating large numbers of putative modifications simultaneously. The methods developed for these biologics are generic and widely applicable to any recombinant product. The development of these approaches and their use in characterization of the proteins will be discussed with particular detail on quantitative differences observed on post translational modified components.

S-06 (P-068)

RNA-binding CCCH-type zinc-finger proteins are essential for oocyte maturation in *C. elegans*

Masumi Shimada[2], Hideyoshi Yokosawa[1], **Hiroyuki Kawahara**[2]

[1]*Department of Biochemistry, Graduate School of Pharmaceutical Sciences, Hokkaido University,*
[2]*Department of Biological Sciences, Tokyo Metropolitan University, Japan*

Oocyte maturation is an important prerequisite for the production of progeny. Although several germ-line mutations have been reported, the precise mechanism by which the last step of oocyte maturation is controlled remains unclear. In Caenorhabditis elegans, CCCH-type zinc-finger proteins have been shown to be involved in germ cell formation, although their involvement in oocyte maturation had not been fully investigated. Using a multiple RNAi technique, we have identified three redundant CCCH-type zinc-finger genes, moe/oma-1, -2 and moe-3, as a group related by functions and nucleotide sequence. Similar results were reported by Detwiler et al (2001). We and Detwiler et al have found that they have overlapping functions that are crucial for oocyte maturation; i.e. simultaneous removal of these proteins by RNAi induces arrest and expansion of growing oocytes. The results of our in situ hybridization have revealed that each of the moe/oma transcripts is expressed from the distal to proximal region of the gonad, while their corresponding proteins accumulate specifically in the cytoplasm of growing oocytes as well as on P granules. Thus, these gene products participate in processes in the final step of the meiotic cell cycle control, a novel function for CCCH-type zinc-finger family proteins. We are interested in the RNA species that are complexed with OMA/MOE-family proteins since CCCH-type zinc-finger is proposed to regulate mRNA metabolism. Our recent progress will be discussed at the conference.

S-07 (P-089)

Analysis of specific proteins related to chemotherapy sensitivities in gliomas by assembled proteomic strategies

Norie Araki[1], Anthony Wilson[1], Takashi Morikawa[1], Nobuyuki Tsubota[2], Megumi Nagayama[1], Daiki Kobayashi[1], Masayo Wilson-Morifuji[1], Uichi Midorikawa[1], Hideo Nakamura[2], Junichi Kuratsu[2]

[1]Department of Tumor Genetics and Biology, Graduate School of Medical Sciences, Kumamoto University, [2]Department of Neurosurgery, Graduate School of Medical Sciences, Kumamoto University, Japan

After developing an assembled proteomic analysis system, we have studied the role of tumor-related gene functions in cancer tissue. In this study, we utilized this system for the analysis of brain tumor-specific proteins related to chemotherapy sensitivities. Malignant gliomas are generally resistant to all standard therapies, however, patients with Anaplastic oligodendroglioma (AOG) with loss of heterozygosity on chromosome 1p/19q (LOH+) frequently respond to chemotherapies. To clarify the mechanism of chemotherapy sensitivity of AOG, we analyzed tissue-specific proteins which show different expressions and phosphorylations in each tumor. Tumors collected from the AOG patients were analyzed by pathological and genomic background and divided into two groups according to chemotherapy sensitivity (LOH+/-). The proteins prepared from each tumor were subjected to 2-D DIGE combined with ProQ diamond staining and iTRAQ. We identified 105 specific proteins expressed in LOH- compared with LOH+, including EGFR-MAPK and PI3K-AKT signal molecules, vascular PIFs, adhesion molecules, cell cycle regulator CDK families, and cytoskeletal organizing factors. Among them, we focused on vimentin molecules that were highly expressed in LOH- samples with specific phosphorylated forms (at least 15 vimentin spots were identified), and confirmed increased expression of vimentin in LOH- compared with LOH+ by immunohistochemistry and 2D-western blotting. In addition to the specific phosphorylation, vimentin had some cleavage forms in LOH-. We classified the modified forms into 4 different MW groups: 53kDa (group1), 48kDa (group2), 45kDa (group3) and 42kDa (group4). By 2D-western blotting and MS analysis, we identified that the spots of group4 and the acidic side of group1 were proteolytic forms and specific phosphorylation forms of the N-terminal vimentin, respectively. In addition, some of the protein spots of groups 1, 3 and 4 of LOH- had much higher expression than those of LOH+. The specific structure and functional changes of vimentin, and the responsible enzymes whose regulation is related to the chemotherapy sensitivity, will be analyzed and discussed in detail.

Young Scientist Talks

Y-01 ~ Y-11

Y-01 (P-078)

Structural flexibility of human nuclear receptor ERRγ to adopt endocrine disruptor bisphenol A and its derivatives

Ayami Matsushima[1], Hiroyuki Okada[1], Xiaohui Liu[1], Takatoshi Tokunaga[1], Takamasa Teramoto[2], Yoshimitsu Kakuta[2], Yasuyuki Shimohigashi[1]

[1]Department of Chemistry, The Research-Education Centre of Risk Science, Faculty and Graduate School of Sciences, Kyushu University, [2]Laboratory of Biochemistry, Department of Agricultural Chemistry, Faculty and Graduate School of Agriculture, Kyushu University, Japan

Bisphenol A (BPA) is a widely used industrial chemical in the manufacture of polycarbonate plastics and epoxy resins and has been recognized as one of the most potent endocrine disruptors. We have recently found that BPA binds much more strongly to estrogen-related receptor γ (ERRγ) than estrogen receptor (ER) [1]. This raises the possibility that BPA's various low-dose effects recognized as endocrine disruptions might be mediated through ERRγ. ERRγ belongs to a subfamily of orphan nuclear receptors, and its intrinsic ligands are still unknown to date. BPA binds to ERRγ with maintaining a high constitutive activity, because, as shown by crystal structure of the ERRγ/BPA complex, ERRγ possesses a ligand binding pocket just for BPA in its active conformation [2]. 4-Hydroxytamoxifen (4-OHT) has been identified as an inverse agonist of ERRγ, which deactivate the receptor by destroying such active conformation. Thus, BPA and 4-OHT are able to displace each other, and the receptor ERRγ changes its conformation from active to inactive forms, depending upon whether or not BPA is a substituent.

BPA has a symmetrical structure, the two phenol groups of which bind to each specific site mainly by hydrogen bondings. We found that, in spite of the lack of one of the BPA-phenols, 4-α-cumylphenol exhibits a strong binding ability to ERRγ as well as BPA [3]. The X-ray crystal structural analysis of the complex revealed that the receptor residue Leu345 manipulates the binding of BPA and 4-α-cumylphenol by rotating its β-isopropyl group for making a strong hydropholic bond with either phenol-benzene of BPA or phenyl of 4-α-cumylphenol. The result clearly indicated that this strong binding of 4-α-cumylphenol is due to the structural flexibility of Leu-side chain, resulting in a formation of the ligand binding in an induced-fit manner [4].

References

[1] S. Takayanagi, et al. Toxicol. Lett. 167, 95 (2006).
[2] A. Matsushima, et al. J. Biochem., 142, 517 (2007).
[3] H. Okada, et al. Environ. Health Perspect. 116, 32 (2008).
[4] A. Matsushima, et al. Biochem. Biophys. Res. Commun. in press.

Y-02 (P-039)

Three-dimensional structure of gastric H/K-ATPase at 6.5 Å resolution determined by electron crystallography of two-dimensional crystals

Kazuhiro Abe, Kazutoshi Tani, Tomohiro Nishizawa, Yoshinori Fujiyoshi

Department of Biophysics, Faculty of Science, Kyoto University, Japan

Gastric H/K-ATPase is a proton pump responsible for gastric acid secretion. The structure of this enzyme is of considerable interest because the million-fold proton gradient which it generates is the largest ion gradient known in mammalian tissues. However, structural information of H/K-ATPase has been missing in contrast to recent progress in structural studies of other P-type ATPase isoforms. Here, we have determined the three-dimensional structure of pig gastric H/K-ATPase at 6.5 Å resolution by cryo-electron microscopy of two-dimensional crystals. The structure includes both catalytic α-subunit and type II membrane protein β-subunit, which is involved in correct membrane integration and routing to the cell surface of the αβ-complex. Although the 2D crystals were grown in the presence of Al^{3+}, F^- and ADP (known as a pseudo substrate for E1P-ADP), the relative orientation of the clearly resolved cytoplasmic domains (A, P and N) and limited proteolysis experiments suggest that the H/K-ATPase is adopted in a pseudo-E2P state. Comparison between our 6.5 Å map and the recently reported Na/K-ATPase atomic model shows that they share considerable similarity, thus allowing us to build homology model of the H/K-ATPase. However, in contrast to the reported structure of Na/K-ATPase, the N-terminal tail of the β-subunit is in contact to the cytoplasmic part of the α-subunit. It is known that H/K-ATPase shows a strong preference for the E2P state, namely, proton-occluded E1P is immediately isomerized to E2P with releasing proton to the luminal side. The observed unique interaction is likely to stabilize E2P conformation, and therefore prevent the interconversion from E2P to E1P. A possible function of this "latchet"-like effect could be the prevention of H^+ reverse-flow to achieve million-fold H^+ gradient across gastric parietal cell membranes.

Y-03 (P-073)

Xyloglucan recognition mechanism suggested from the crystal structure of Cel44A

Yu Kitago[1], Shuichi Karita[2], Nobuhisa Watanabe[3], Kazuo Sakka[2], Isao Tanaka[1]

[1]Faculty of Advanced Life Science, Hokkaido University, [2]Major of Sustainable Resource Science, Graduate School of Bioresources, Mie University, [3]Graduate School of Engineering, Nagoya University, Japan

Some microorganisms were confirmed to produce the huge multiprotein complex called the cellulosome that has the plant cell-wall degradation activity. This complex consists of the noncatalytic scaffolding protein and the various enzymatic proteins. The cellulosomal enzymatic proteins contain many kinds of cellulases such as endoglucanases, xylanases, mannanases, cellobiohydrolases, glucosidases, etc. This variety of enzymatic proteins makes it possible to degrade crystalline cellulose quite efficiently. In these enzymatic proteins, enzymes that belong to glycoside hydrolase family (GH family) 44 appear in some celulolytic bacteria's cellulosome, and those had been only known as its endoglucanase, lichenase, xylanase and xyloglucanase activity.

This study aims to reveal the detailed structure and mechanisms of the GH family 44 enzymes by X-ray crystallographic study of Cel44A from *Clostridium thermocellum* , which is known as the major anaerobic cellulolytic bacteria that produce the cellulosome. The multidomain endoglucanase CelJ is the biggest enzymatic component of *C. thermocellum*'s cellulosome, and Cel44A is one of the enzymatic module of CelJ. Previously, Cel44A was cloned and confirmed its cellulolytic ability. In this study, Cel44A crystal structure was determined for the wild-type and mutant Cel44A with two types of substrates. The revealed structure shows the substrate recognition mechanisms, and suggests that two glutamic acid residues, Glu186 and Glu359, work as catalytic residues. The catalytic ability of these two residues is confirmed by biological assay of each mutant, E186Q and E359Q. The structural features suggest that Cel44A has the catalytic mechanism of retention that is contrary to the previous report. From the ^{1}H-NMR chronological experiment against Cel44A activity, the direct evidence for the mechanism of retention was obtained. On the molecular surface, some side cleft and pockets of the catalytic cleft were observed. Interestingly, the xyloglucanase activity of Cel44A was reported, and this architecture seems to be reasonable for the recognition of xyloglucan.

Y-04 (P-049)

New approaches for developing therapeutic drugs against neutrophil activation by HPNAP from Helicobacter pylori

Filippos Kottakis[1], Georgios Papadopoulos[3], Christina Befani[1], **Antonios Asiminas**[1], Georg Koliakos[2], Theodora Choli-Papadopoulou[1]

[1]Aristotle University of Thessaloniki, School of Chemistry, Laboratory of Biochemistry, [2]Aristotle University of Thessaloniki, Medical School , Laboratory of Biological Chemistry, [3]Department of Biochemistry and Biotechnology, University of Thessaly, Larissa, Greece

The Helicobacter Pylori Neutrophil Activating Protein (HP-NAP) was originally purified from water extracts of H. pylori and was shown to induce neutrophil adhesion to endothelial cells *in vitro* and *in vivo*. HP-NAP protects DNA from free radicals as a dodecamer through its ferroxidase activity without, however, directly binding to it. DNA protection in environments rich in free radicals, ferroxidase activity and dodecamer formation abilities were abolished after replacement of four out of five residues located in the ferroxidase site (His25, His37, Asp52 and Lys134 to Ala). Molecular dynamics simulations revealed that dimer formation is highly unlikely following mutation of the above mentioned amino acids, as the Fe2+ is no longer attracted with equal strength by both subunits. These findings probably indicate that iron plays an important role in the conformation of HP-NAP by initiating the formation of stable dimers that are indispensable for the ensuing dodecamer structure. In an attempt to investigate HPNAP's role in neutrophil activation, the amino- terminal 1-57 and carboxy- terminal 58-144 regions of the protein were cloned, expressed and purified. Neutrophil activation assays with the HPNAPwt, HPNAP1-57, HPNAP58-144 and HPNAPmut demonstrated that the dodecamer conformation does not seem to be necessary for activation, and helices H3 (Leu69-Leu75) and H4 (Lys89-Leu114) or the linking coils (His63-Thr68 and Thr76-Ser88) found in the C-terminal HPNAP58-144 are probably critical in stimulating neutrophil activation .These results were supplemented by detection and quantification of lipopolysaccharides (LPS) in the above mentioned proteins. Even though these experiments indicated that the LPS level in all protein preparations were below the neutrophil activation threshold, the endotoxins were removed using Polymyxin B coated magnetic beads. The LPS-free proteins were used to study their effect on the adhesion of neutrophils to endothelial cells utilizing the myeloperoxidase (MPO) assay.

Y-05 (P-070)

Structural and functional analysis of a novel, highly conserved, glomerulus specific protein shisa3

Juha R.M. Ojala[1], Zhijie Xiao[1], Jaakko Patrakka[1], Christer Betsholtz[2], Karl Tryggvason[1]

[1]*Department of Medical Biochemistry and Biophysics, Division of Matrix Biology,* [2]*Vascular Biology, Karolinska Institutet, Sweden*

To gain more understanding of the function and diseases in the glomeruli, the filtration units of the kidney, 300 glomerular cell-enriched transcripts were identified in a large-scale microarray analysis. Within this list of genes, a previously uncharacterized type I membrane protein with high homology between species and to several other unknown proteins was identified. Later the protein was found to be the third member of a shisa protein family. The high conservation indicates important function for the protein, and interestingly, within shisa3 sequence the most conserved extracellular portion of the protein does not seem to resemble any other folds in the protein data bank. Therefore, a gene knockout and protein crystallography approaches have been initiated.

After the initial microarray data the expression of shisa3 in the kidney glomeruli was confirmed with *in situ* hybridization. Northern blot, RT-PCR, and immunohistochemistry from multiple tissues revealed the expression also in the testis, heart, and brain. Next the knockout mice were generated so that the entire coding region was replaced with a cassette containing the neomycin resistance gene and the green fluorecent protein gene. The mice are born without any apparent phenotype, but the careful analysis of especially the kidney function remains to be done.

For structure analysis, the 75 residues long extracellular part of shisa3 containing eight cysteines was produced first in the insect cells and later in the mammalian cells. Interestingly, all the attempts to produce antibodies against shisa3 purified from the insect cells failed in contrast to the protein produced in the mammalian cells. This is most probably due to the lack of proper O-glycosylation within the insect cells. In fact, as analyzed by mass spectrometry, the protein from the insect cells was purified in two different glycosylation states while totally eight forms could be separated from the mammalian cells. Thus far, one of the insect cell-produced forms of the protein has been successfully crystallized and a native X-ray diffraction dataset collected to 1.4 Å resolution.

Y-06 (P-054)

Alteration of *Streptomyces clavuligerus* deacetoxycephalosporin C synthase substrate binding pocket to accommodate penicillins with aromatic side chains

Kian Sim Goo, Tiow-Suan Sim

Department of Microbiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

The industrial production of cephalosporin nucleus is currently done based on chemical ring-expansion of penicillin G. However, this method is expensive and polluting. In *Streptomyces clavuligerus*, its natural substrate penicillin N is converted to a cephalosporin by deacetoxycephalosporin C synthase (DAOCS). The prospect of using DAOCS in an enzymatic process is an attractive one and has warranted studies to improve its substrate specificity to accommodate readily available penicillins with aromatic side chains, e.g. penicillin G and ampicillin, instead of the aliphatic penicillin N. With the recent availability of crystal structures of DAOCS complexed with penicillin G and ampicillin, using the SwissPdbViewer reveals amino acid residues coordinated to the respective substrates within a selected distance. Thus, amino acid residues M73, S102, L158, R160, R162, F264, N304 and I305 were identified to be interacting with penicillin G and ampicillin within 5Å radius. Of these, M73 was found to be in close proximity to the aromatic side chains of these two penicillins. The LIGPLOT and Ligand-Protein Contacts prediction programmes were also employed to access the interactions between the penicillin substrates and to deduce strategic amino acid residues involved. Both analyses have also pointed to positive interactions between the penicillin substrates and the amino acid residue at position 73. Kinetic studies have indicated that DAOCS can convert penicillin G and ampicillin. Also, when M73 was modified to a smaller amino acid residue threonine, the substrate binding affinity of DAOCS improved by about 4-fold. Based on these notions, we decided to investigate, *in silico*, the prospect of altering the substrate binding pocket of DAOCS at the M73 position. Analyses based on using the SwissPdbViewer suggested that the molecular size, hydrophobicity and charges of certain amino acids may influence the enzyme's prowess. These predictions will be subjected to validation by experimentation.

Y-07 (P-050)

Toll-like receptors 2 and 4 can be activated by vEP protease to induce an inflammatory response

Jung Eun Park, Nam Hee Lee, Jong Woo Park, Jae Yeong Park, Jung Sup Lee

Department of Biotechnology, BK21 Research Team for Protein Activity Control, and Research Center for Proteineous Materials, Chosun University, Gwangju 501-759, Korea

We have purified and characterized a broad-specificity metalloprotease designated to as vEP secreted by *Vibrio vulnificus* ATCC29307. vEP is capable of cleaving a variety of plasma proteins which are associated with blood clotting (Chang *et al*., J. Bacteriol. 187: 6909-6916, 2005). The protease can be inhibited by its own N-terminal propeptide (Chang *et al*., J. Bacteriol. 189: 6832-6838, 2007). Recently, we found that vEP could mediate the production of pro-inflammatory cytokines such as TNF-alpha and IL-1beta in a macrophage cell line, in which NF-kappaB signaling pathway was activated through the degradation of IkappaB, as judged by ELISA, RT-PCR, real-time PCR, Western blotting, EMSA, and super-shift assay. Among the Toll-like receptors TLR-2 and -4 were activated by vEP, as determined by RT-PCR, immunoprecipitation, Western blotting, and confocal imaging. These results suggest that vEP can induce inflammatory response by activating TLR-2 and -4 that lead the translocation of NF-kappaB into nucleus to activate the target genes such as TNF-alpha-encoding gene. Increase in NO production and Cox-2 expression by vEP further supported the fact that vEP can act as an inflammation inducer. Interestingly, a deletion mutant enzyme of vEP deficient in C-terminal 100 amino acids (called C-ter100 or C-domain) failed to show those kinds of response at all. However, C-ter100 only was sufficient to produce TNF-alpha, suggesting that the C-domain of vEP may play a critical role in the induction of inflammatory response by activating the Toll-like receptors. Taken together, the results obtained by this study suggest that the production of extracellular protease(s) from Vibrio can disturb blood homeostasis as well as induce inflammation during the bacterial infection.

Y-08 (P-47)

An alternative splicing variant of the PPM1 protein phosphatase PPM1D

Yoshiro Chuman, Wataru Kurihashi, Yohei Mizukami, Takehiro Nashimoto, Hiroaki Yagi, Kazuyasu Sakaguchi

Department of Chemistry, Faculty of Science, Hokkaido University, Japan

PPM1D (Wip1, PP2Cδ) is a member of the PPM1 type (former as PP2C) protein phosphatases and was originally identified as a p53-inducible protein phosphatase in response to DNA damage by ionizing radiation. PPM1D-deficient mice show defects in spermatogenesis and lymphoid cell functions but the mechanisms underlying these phenotypes remain unknown. In this study, we identify and characterize an alternative splicing variant, PPM1D430, of human PPM1D at both the mRNA and protein level. PPM1D430 comprises the same 420 residues of the standard full length protein (PPM1D605) and in addition contains a stretch of 10 amino acids that are PPM1D430-specific and are derived from a coding region of 111 bp between exon 5 and exon 6. Semi-quantitative RT-PCR analysis revealed that PPM1D430 mRNA is also induced in response to the genotoxic stress in a p53-dependent manner. On the other hand, expression profiling of this gene by RT-PCR analysis of a human tissue cDNA panel revealed that PPM1D430 is expressed exclusively in testes and in leukocytes whereas PPM1D605 is ubiquitous. In addition, PPM1D430 shows a different subcellular localization pattern and protein stability when compared with PPM1D605 under some conditions. *In vitro* phosphatase analysis and PPM1D430-specific RNA interference analysis further indicated that PPM1D430 can dephosphorylate Ser15 of human p53 both *In vitro* and *In vivo*. Our current findings thus suggest that PPM1D430 and PPM1D605 may exert diverse functions through their different properties in cellular distribution, subcellular localization and protein stabilities.

Y-09 (P-058)

Landscaping enzymatic reactions of oxidoreductases by a self-organizing neural network

Keisuke Ueno, Katsuhiko Mineta, Toshinori Endo

Graduate School of Information Science and Technology, Hokkaido University, Japan

With the availability of larger enzyme structure database, structure-based methods to recognize the conserved orientations of functional groups and ligand-binding patterns become more attractive. Especially, how the physicochemical properties represent the enzyme functions is of great interest because the structure-based function predictions only from the visual inspection of structural models are fragile for the conformational changes and for ambiguous functional groups. Here we introduce a new approach by which the physicochemical properties of catalytic sites determine the enzyme functions. Toward the elucidation of the enzyme functions, the high-dimensional physicochemical variables of catalytic sites of oxidoreductases were mapped onto two-dimensional space using the Kohonen neural network. Landscaping the enzymatic reactions of oxidoreductases shows the enzyme commission numbers are clearly divided. We found that the cluster formation is strongly affected by the hydropathy, which plays a key role in enzymatic reactions due to transfer of charge such as protons and electrons between enzymes and ligands. The profile of catalytic residues was also mapped onto the landscape. The characteristic residues of the catalytic sites are similar among the same cluster and gradually vary the profile by distance on the map. Furthermore, we found that smoothing the data space shows the stability of the cluster. It is likely that the smoothing factor is analogous to the optimal temperatures of enzymatic reactions. Thus, the physicochemical properties of catalytic sites represent the enzyme functions, supporting the findings that the physicochemical properties of ligands reveal enzyme functions. Compared to the solely sequence- or structure-based method, this method is useful in implicitly combining these properties. This method can be applied to mimicking the enzymatic reactions and predicting the enzyme functions. Although this method is susceptible to conformational changes due to the properties based on the orientations from the reaction centers, we can detect the abnormality of the structures such as mutations or conformational changes.

Y-10 (P-032)

Single molecule dynamics studied with atomic force microscopy: The effect of temperature on the energy landscape of mechanical unfolding of proteins

Yukinori Taniguchi[1], Masaru Kawakami[1,2]

[1]*School of Materials Science, Japan Advanced Institute of Science and Technology (JAIST)*
[2]*PRESTO of Japan Science and Technology Corporation (JST), Japan*

Atomic force microscopy (AFM) is one of the most useful techniques for studying the mechanical properties of proteins at the single molecule level. A single protein molecule attached between the AFM cantilever tip and the substrate is stretched at a constant pulling speed, which provides the force-extension curve and unfolding forces. The force-extension curve reflects the character of proteins such as the number and size of folded domains, the mechanical resistance and unfolding pathway, *i. e.* it provides the mechanical fingerprint of each protein. Using this technique, we have studied the mechanical unfolding of I27 domain of human cardiac titin and tail domain of rabbit skeletal myosin II (myosin rod). The mechanical stability of these proteins is believed to be important for their function in muscle contraction. In this paper we have investigated the effect of temperature on the mechanical stability.

I27 has a β-sandwich structure and unfolds by a three-state mechanism (N→I→U). Reflecting the partial unfolding from the native state (N→I) a hump structure is seen in the force-extension curve. Interestingly, the force at the partial unfolding was unaffected in the temperature range of 2-30 °C. On the contrary, the force at the catastrophic unfolding from the intermediate state (I→U) showed a remarkable increase at lower temperatures. A kinetic analysis showed that the effect of temperature was explained by the change of the distance between the intermediate state and the transition state in the energy landscape. Additionally, we estimated the roughness of the energy landscape to be 4.3 k_BT. In the case of α-helical coiled-coils of myosin rod, the coiled-coil is unravelled by force upon stretching and results in a plateau in its force-extension curve. The plateau force was ~50 pN, independently of temperature. These results indicate that the effect of temperature on the mechanical resistance of the two proteins is distinct from each other, reflecting the difference in the mechanism stabilising their structures. We conclude that local unfolding reaction of topologically-simple structure is hardly affected by temperature.

Y-11 (P-007)

Selective extraction and enrichment of multi-phosphorylated peptides using polyarginine-coated diamond nanoparticles

Chih-Che Wu[1], Chia-Kai Chang[1], Yi-Sheng Wang[2], Huan-Cheng Chang[3]

[1]Department of Applied Chemistry, National Chi Nan University, [2]Genomics Research Center, Academia Sinica, [3]Institute of Atomic and Molecular Sciences, Academia Sinica, Taiwan

Despite recent advances in phosphopeptide research, detection and characterization of multiply phosphorylated peptides have been a challenge. This work presents a new strategy that not only can effectively extract phosphorylated peptides from complex samples but also can selectively enrich multi-phosphorylated peptides for direct matrix-assisted laser desorption/ionization time-of-flight mass spectrometric analysis. Polyarginine-coated diamond nanoparticles are the solid-phase extraction supports used for this purpose. The supports show an exceptionally high affinity for multi-phosphorylated peptides due to multiple arginine-phosphate interactions. The efficacy of this method was demonstrated by analyzing a small volume (50μL) of tryptic digests of proteins such as alpha-casein, beta-casein, and non-fat milk at a low concentration. The concentration is markedly lower than that can be achieved by using other currently available technologies. We quantified the enhanced selectivity and detection sensitivity of the method using mixtures composed of mono- and tetra-phosphorylated peptide standards. This new affinity-based protocol is expected to find useful applications in characterizing multiple phosphorylation sites on proteins of interest in complex and dilute analytes.

Poster Session

P-001

Analysis of lactate dehydrogenases (LDHs) from hagfishes in high pressure conditions by high-pressure electrophoresis and high-pressure photometry

Yoshikazu Nishiguchi[1], Fumiyoshi Abe[2], Akira Uchida[3], Tetsuya Miwa[2], Chiaki Kato[2], Takako Sato[2], Souichirou Kubota[3], Yasui Kinya[4], Noriko Oshima[3], Hiroyuki Satoh[3], Muneo Morishita[1], Michiko Goromaru-Shinkai[1], Nobue Ito[3], Mitsumasa Okada[3]

[1]Faculty of Pharmaceutical Sciences, Toho University, Japan, [2]Extremobiosphere Research Center, JAMSTEC, Japan, [3]Faculty of Science, Toho University, Japan, [4]Department of Biological Science, Hiroshima University, Japan

Hydrostatic pressure is thought to have the greatest influence on the vertical distribution and speciation of organisms in the deep seas and on the formation of protein complexes, e.g., enzyme-substrate or protein-protein interactions. However, the primary structures of those proteins have not been determined in detail. In this study, we compared the kinetic properties and amino acid sequences of LDH-A_4 from three hagfishes and found pressure-adaptive changes in LDH-A_4 structure and function. The effect of high hydrostatic pressure on LDH activity was examined from 0.1 to 100 MPa. The activity of LDH-A_4 from *E. burgeri*, which inhabits the shallow sea, was lost at pressure greater than 5 MPa. The activity of LDH-A_4 from *P. atami*, living at around 400 m, did not change at pressure of 10 MPa, but decreased to about 55 % at 15 MPa and to about 15 % at 20 MPa. Among the three hagfishes, *E. okinoseanus* has the deepest habitat at around 800–1000 m and it showed the greatest pressure resistance. Its LDH activity was constant up to pressure of 40 MPa and remained at 70 % even at 100 MPa. These results suggest that LDH-A_4 from *E. okinoseanus* has a structure with the greatest adaptation to pressure among the genera and species examined. There were differences in six amino acid residues (6, 10, 20, 156, 269, and 341) when comparing the LDHs of the three hagfishes. Four of the amino acid residues (6, 10, 20, and 341) occur where the four monomers combine to form tetramers and two amino acid residues (156 and 256), which are in the neighborhood of the active site, may control enzymatic activity. To investigate the effect of high hydrostatic pressure on the stability of subunit formation of LDHs, the electrophoresis was performed under 50 MPa, using the high-pressure electrophoresis apparatus. At this pressure, the activity of LDH-A_4 from *E. burgeri* was completely lost, although that from *E. okinoseanus* was maintained. The electrophoresis pattern of *E. okinoseanus*-$LDHA_4$ was only one band near the cathode indicating that the LDH-tetramers were intact at 50 MPa. In *E. burgeri* there were one faint band near the cathode and two broadened bands migrated to the anode. The LDH-tetramers of *E. burgeri* were almost completely dissociated to dimmers and monomers which were known to be inactive. The result explains the cause of the inactivation of *E. burgeri* -LDH at relatively low pressure. The mechanism of the inactivation of *E. okinoseanus*-LDH at high pressure may be different, probably the metamorphosis of the inner structures.

P-002

Rapid, comprehensive and high-resolution intact protein separation for proteomics

Angelika Koepf [1], Matsamitu Okawara [2], Karl Burgess [3], Ken Cook [4], Andrew Pitt [3]

[1] Dionex Corp., Asia Pacific, Bangkok, Thailand, [2] Nippon Dionex K.K., Tokyo, Japan, [3] University of Glasgow, Glasgow,, [4] Dionex Corp., Camberley, UK

Introduction: Sample complexity is one of the key challenges facing proteomic analysis. New developments in column technology allow us to perform rapid improved-resolution MS based identification of intact proteins from complex samples. Sample types investigated include bacterial lysates (Bordetella parapertusis, and E. coli), a eukaryotic parasite (Leishmania donovani), and transformed human cell lines.
Method: We report the separation of complex protein mixtures using online 2D LC on PSDVB pellicular IEX resins and PSDVB monolithic RP columns with proteolytic digestion of the fractions followed by rapid LC-MSMS. An alternative methodology, relying on direct analysis of the second dimension eluents by top-down methodology, using the Apex IV 12T FTICR-MS has allowed identification from intact Leishmania proteins and PTM mapping of histone H4. Separation of a typical amount of lysate (200ug) was performed using anion exchange columns, followed by RP separation using rapid gradients on a 500um PS-DVB monolith. Fractions (20uL) from the second dimension were collected in 384 well microtitre plates and subjected to trypsin digestion. Results: The use of parallel 200um monoliths for tryptic peptide separations ensured maximum capacity, minimum sample loss and high sample throughput, with no loss of sensitivity. For simple mixtures, RP separation times could be reduced to a few minutes without significantly affecting data content, although rapid scanning capability was essential due to the very narrow peak widths. Analysis of the digested fractions gave good coverage of the proteome. Proteins representing low (8kDa) and high (500kDa) molecular mass and extremes of predicted pI were identified, as well as a number of membrane proteins. Resolution of the intact protein separation was such that single protein species often occurred in one or two fractions for both the IEX and RP separations. Separation of modified proteins in the IEX dimension demonstrated separation of isoforms. UV absorbance maps were generated for quantification and could be used for differential analysis of samples. In addition, isotopic labelling techniques have been employed for more in-depth analysis. Additionally, label-free techniques have been employed for protein quantitation by LC/FT-ICR-MS.

P-003

An efficient screening method for protein-binding peptides using capillary electrophoresis-mass spectroscopy (CE-MS)

Kazuki Saito[1], Mamiko Nakato[2], Takaaki Mizuguchi[2], Hiromasa Uchimura[1], Shigeyuki Yokoyama[3], Hiroshi Hirota[3], Yoshiaki Kiso[2]

[1]Laboratory of Proteomic Sciences, 21st Century COE Program, Kyoto Pharmaceutical University, [2]Department of Medicinal Chemistry, Center for Frontier Research in Medicinal Science and 21st Century COE Program, Kyoto Pharmaceutical University, [3]Protein Research Group, RIKEN Genomic Sciences Center, Japan

Recent clinical proteomic studies have revealed a variety of pathogenic proteins, and the discovery of drugs against such disease-causing proteins has emerged as one of the hot-topics for proteome researchers. Previously, as a first step toward discovery of drug lead compounds, we developed a general strategy for screening small organic compounds that bind to proteins [1, 2]. In the strategy, based on the 3-D structure of the target protein, ligand candidates are selected by computer screening from a library of compounds, and are then verified for their affinities with the protein by surface plasmon resonance measurements. However, the strategy cannot be applied to targets whose structural information is unavailable, and, therefore, much more general strategies for ligand screening should be explored as alternatives. In this study, an efficient strategy to discover peptides that bind to a target protein has been developed using capillary electrophoresis (CE) in combination with mass spectrometry (MS). Peptides would be excellent leads for development of inhibitors, since a target protein usually exhibits its activities through binding to another partner protein and it may recognize partial peptide sequences of the partner.

In experiments using calmodulin as a model target, the strategy was revealed to require only small amount (~15 μL) of the target protein solution, and be able to detect even weak interactions up to sub-millimolar K_d values by adjusting the concentration of the solution. In fact, several calmodulin-binding peptides were successfully yielded out of a mixture, which consists of more than 50 peptide fragments, prepared with an endoproteinase digestion of reduced bovine serum albumin (BSA). Our screening methodology with CE-MS is expected to be a versatile approach to discover inhibitory peptides against medicinal target proteins without any structural information.

References

[1] Yoshitani *et al.*, *Proteomics*, **5**, 1472–1480 (2005).
[2] Yoshitani *et al.*, *Proteomics*, **7**, 494–499 (2007).

P-004

Using a mapping function into a higher-dimensional space for filtering criteria against SEQUEST database search results

Kouki Yonezawa [1], Katsuhiko Mineta [1], Toshinori Endo [1], Lixy Yamada [2], Hisaaki Taniguchi [2]

[1] *Graduate School of Information Science and Technology, Hokkaido University,* [2] *The Institute for Enzyme Research, The University of Tokushima, Japan*

Recently efficient and accurate methods for protein identification are becoming more and more important especially for a huge amount of the data produced by the tandem mass spectrometry (MS/MS). Each MS/MS spectrum is identified by utilizing a database search algorithm, such as SEQUEST, a representative protein identification algorithm. However, SEQUEST does not give sufficient confidence scores on the search result and so we need some criterion in order to evaluate the correctness of the result. So far several methods have been proposed to achieve filtering criteria that achieve the false positive ratio (FPR) close to the given FPR by a user in advance. There are mainly two difficulties. First, there is a tradeoff between achieving the better FPR and getting the higher sensitivity. Second, the spreads of correct and incorrect data overlap in a significant part. Here, we propose the novel approach for achieving filtering criteria. Our intuitive idea is as follows: ``If the given data has higher dimensions then it can be distinguished more easily". Our approach uses a mapping function that maps the given data into a higher-dimensional space, which is the primitive idea of kernel machines. Then we calculate a plane to distinguish the higher-dimensional space that gives an FPR close to the given one for the training dataset. It should be noted that our algorithm can treat more scores calculated by SEQUEST, for example, Sp and SpRank. In order to demonstrate the efficiency of our approach, we compared the result of our algorithm and those of several algorithms proposed so far. We prepared two dasesets for our analysis. One is a set of spectra of [Purvine et al., 1994], and another is a set of spectra given by the Ciona Intestinalis. As a result of the analysis, we showed that the two scores XCorr and deltaCn are enough for our algorithm to give a criterion, and from the comparison with the traditional algorithms, our algorithm achieves the closer difference by more than 10% between the given FPR and the actual one.

P-005

Multiple MS fragmentation as a versatile tool for discrimination of glycan isomers: Application to the neural glycoprotein CD24

Christina Bleckmann[1], **Hildegard Geyer**[1], Ralf Kleene[2], Melitta Schachner[2], Vernon Reinhold[3], Rudolf Geyer[1]

[1]Institute of Biochemistry, Faculty of Medicine, Justus-Liebig-University Giessen, [2]Center of Molecular Neurobiology, University of Hamburg, [3]Division of Molecular, Cellular, & Biomedical Sciences, University of Hampshire, Germany

Structural elucidation of complex carbohydrate molecules is difficult because it requires the knowledge of several parameters such as sugar constituents, sequence, branching, linkage type between the monosaccharides, and anomeric configuration. In recent years, mass spectrometry (MS) has gained increasing importance in this field. In particular, electrospray ion trap ionization (ESI-IT-) and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF-) MS have been shown to represent powerful techniques in the context of carbohydrate analysis.The neural glycoprotein CD24 is extensively glycosylated and has a peptide core of only 27 amino acids. Nearly half of the amino acids represent Asn, Thr and Ser residues that are potential sites for N- and O-glycosylation. Posttranslational modifications, in particular its glycosylation, appear to play an important role in the physiological functions of CD24. Western Blot analyses using lectins and antibodies against different carbohydrate-epitopes revealed the presence of carbohydrate-bound Lewis^x epitopes, HNK-1 determinants and alpha2,3-linked sialic acid. In the present study we have analyzed CD24 N-glycans in more detail. N-glycans were released by PNGaseF treatment and native glycans as well as pyridylaminated species were characterized by MALDI-TOF(/TOF)-MS whereas reduced and permethylated glycans were studied by ESI-IT-MS^n. The results revealed the presence of complex type N-glycans carrying, in part, core-linked fucose, outer Le^x-determinants, H-antigen units, bisecting N-acetylglucosamine and N-acetyllactosamine repeating units. Structural isomers could be defined in many cases without prior chromatographic separation. Interpretations of the obtained, in part, highly complex MS spectra was greatly facilitated by the use of GlycoPeakfinder and GlycoWorkbench, i.e., new software tools generated within the EuroCarbDB design study. Examples of the application of these tools will be presented.

P-006

Protein sequencing analysis using MALDI-TOF-MS and chemical modifications of tryptic peptides

Tomohiro Araki[1], Koichi Sakakibara[1], Atsushi Matsuoka[1], Norie Araki[2]

[1]*School of Agriculture, Tokai University,* [2]*Graduate School of Medical Sciences, Kumamoto University, Japan*

For protein identification, MALDI TOF-MS allows high-sensitivity and high-throughput analysis of peptides. However, compared with ESI MS, MALDI TOF-MS is not a conventional method for de novo sequencing in spite of higher sensitivity than ESI MS due to the complexed fragment ions after MS/MS analysis. In this study, we established an optimized protocol for de novo sequencing to overcome the defects of MALDI TOF-MS. This study is based on the evaluation of several chemical modification methods of tryptic peptides from gels, and aims to establish the optimal conditions for de novo sequencing. After in-gel digestion with trypsin, positive charge was introduced into the C-terminal lysine amino group to emphasize the ionization of lysine peptides, and negative charge into N-terminal amino group to enhance the y-series ions in MS/MS analysis. The results confirmed that the strong positive charge at the C-terminal of a peptide enhanced the ionization in TOF-MS analysis, and MS/MS analysis showed the enhanced y-ion formation. The introduction of negative charge at the N-terminal suppressed the formation of b-ions by neutralizing the positive charge at the C-terminus. To optimize the modification method, the combinations of C-terminal and N-terminal modifications were compared. The optimum combination of two-step modification of the C-terminal lysine amino group and the N-terminal amino group was guanidination followed by SIPTC sulfonation, which enables the sequencing of peptides without any noise signals such as b-ions. This method was also applicable to transblotted proteins on PVDF membrane. Further, the pyridyl ethylation of disulfide bonds prior to in-gel digestion, followed by the combination of guanidination and SPITC modifications was evaluated to establish the protein de novo sequencing method. This established de novo sequencing method was applied to sequencing analysis of HSA and determined 239 amino acid residues out of the total sequence (40.85%) by single TOF-MS analysis.

P-008

Identification and characterization of novel human ORC binding proteins

Masahiro Yamaguchi[1], Satosi Ohta[3], Takasi Seki[1], Koji Nagao[2], Chikasi Obuse[1]

[1]Faculty of Advanced Life Science Hokkaido University, [2]G0 Cell Unit, Initial Research Project, Okinawa Institute of Science and Technology, [3]Structural Biochemistry, Jichi Medical University, Japan

ORC(Origin Recognition Complex) is a conserved protein complex that is essential for formation of the replication initiation complex. In human cells, ORC1 accumulates in G1 phase and forms a functional complex with ORCs 2-5. Recent studies suggest that ORC also has a role in order cellular functions, such as heterochromatin assembly, chromosome condensation and segregation. In order to elucidate the molecular link between ORC and other cellular events, we are characterization ORC binding proteins.Immunoprecipitates containing Flag-tagged ORC1 were purified from nuculear extracts of stably transformed 293 cell line and examined by mass spectrometry.As expected, ORC2, ORC3, ORC4 and ORC5 efficiently co-immunoprecipitated with ORC1. In addition to these ORC subunits, constituents of the telomere (TRF2, Rap1), nuclear membrane (Sad1/Unc84) and an uncharacterized WD40 protein (designated as ORCBP1) were identified. In both the 293 cell line and HeLa cells, the amount of other ORC subunits, implying a tight and stoichiometric association of ORCBP1 with the ORC complex. The above results were confirmed in reciprocal experiments using a HeLa cell line expressing Flag-ORCBP1, in which Flag-ORCBP1 immunoprecipitates contained all ORC subunits. Immuno-fluorescence analyses showed Flag-ORCBP1 localized in nuclei. Moreover, RNAi inhibition of ORCBP1 resulted in a prolonged G1 phase in HeLa cells, demonstrating that ORCBP1 may have a role in coordinating ORC function for correct cell-cycle progression.

P-009

A mechanism for heme-mediated oxidative modification of iron response regulator

Chihiro Kitatsuji[1], Atsushi Nakamura[2], Masaki Kurogochi[3], Shin-Ichiro Nishimura[3], Mark R. O'Brian[4], Koichiro Ishimori[2]

[1]Graduate School of Engineering, Kyoto University, [2]Graduate School of Science, Hokkaido University, [3]Graduate School of Life Science, Hokkaido University, [4]Department of Biochemistry, State University of New York at Buffalo, USA

Iron is essential for life, but the iron availability depends on the environment. Most organisms have various iron-regulated systems. In a nitrogen fixing bacterium, to prevent accumulation of the heme precursor porphyrin under iron limitation, a transcription factor Irr (iron response regulator) blocks transcription of the gene encoding a porphyrin biosynthetic enzyme. Under normal iron availability, Irr is oxidized and inactivated in a unique mechanism that is initiated by the binding of heme to Irr, resulting in resumption of the porphyrin synthesis. In such oxidation mechanism, Irr-bound heme could activate dioxygen to reactive oxygen species (ROS) with reductant, and ROS would oxidize some residues of Irr, inducing inactivation of Irr.To identify ROS, here we performed the oxidation reaction of Irr with some ROS scavengers. Though scavengers for superoxide anion or hydroxyl radical did not affect the oxidation of Irr, hydrogen peroxide scavenger, catalase, markedly reduced the oxidation of Irr. However, hydrogen peroxide is moderate ROS which cannot oxidize amino acids, and more activation of hydrogen peroxide would be required. The hydrogen peroxide activation reaction is often found in proteins bearing non-heme ferrous ions, which allows us to speculate that Irr has non-heme iron. As expected, atomic emission spectrometry showed intrinsic iron ion in Irr, suggesting that hydrogen peroxide is activated by the non-heme iron in Irr to oxidize own peptide. We have not yet determined the oxidation sites of Irr, mass spectrometry clearly indicates that His63 is one of the oxidation sites in Irr. Based on the sequence homology with a hydrogen peroxide-sensor protein PerR, His63 can be one of the ligands for non-heme iron and oxidation of His63 would result in the dissociation of the non-heme iron and destabilization of the protein structure. Thus, we proposed the two-step dioxygen activation in Irr; Irr-bound heme generates hydrogen peroxide using dioxygen and reductant, and hydrogen peroxide is more activated to highly reactive species, which would oxidize His63, one of the key residues for ligation of the non-heme iron and stability of the protein structure.

P-010

Facile MALDI-MS analysis of neutral glycans in NaOH-doped matrices: Microwave-assisted deglycosylation and one-step purification with diamond nanoparticles

Yan-Kai Tzeng, Cheng-Chun Chang, Chau-Chung Han, Huan-Cheng Chang

Institute of Atomic and Molecular Sciences Academia Sinica, Taiwan

A protocol has been developed to accelerate and simplify matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) mass spectrometry (MS) of neutral underivatized glycans released from glycoproteins. It involved microwave-assisted enzymatic release of glycans, followed by rapid removal of proteins and peptides with carboxylated/oxidized diamond nanoparticles, and finally treating the analytes with NaOH before mixing with matrix to suppress the formation of both peptide and potassiated oligosaccharide ions in MS analysis. The advantages of this protocol were demonstrated with MALDI-TOF-MS of N-linked glycans released from ovalbumin and ribonuclease B.

P-011

Effective recovery of bioactive protein from denaturated form using microfluidic channel

Hiroshi Yamaguchi, Masaya Miyazaki, Hideaki Maeda

Nanotechnology Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Japan

Most proteins which have difficulty in folding form inclusion bodies upon expression. These inclusion bodies are subjected to solubilization and subsequent refolding methods, i.e., dialysis, in order to recover the bioactive form. Many successful results were obtained by this procedure, however, the method is not universally applicable because of the lack of sufficient data on the kinetics of removal of detergents and denaturating agents. Microfluidic reaction system is a superior analytical device that enables strict control of solution flow. Mixing of different solutions can be controlled by using efficient microfluidic design. In addition, the system eases kinetic analysis of chemical reactions because the fluidic system is characterized as laminar and therefore, reaction time can be manipulated by the channel length. Studies of protein folding kinetics using microchips have been reported, however, there is no study to recover bioactive proteins from denaturated proteins. In the present study, we examined the effect of dilution process on protein refolding. Urea-denaturated citrate synthase (CitSyn), which is recognized difficult-to-refold protein, was used as a model.

To examine the effect of dilution process on protein refolding, we designed two microfluidic chips, MR1 and MR2. MR1 directly dilutes the sample, while MR2 involves a multi-dilution step. The refolded CitSyn by MR1 showed the same secondary structure and enzymatic activity as those of the batch sample. In contrast, the CitSyn prepared by MR2 with a multi-dilution (2-fold to 5-fold) showed higher helical structure and activity compared with those of the batch protein. This protein refolding process was achieved within a few minutes. Moreover, mild dilution (2-fold to 5-fold) is a better refolding process than rapid dilution (5-fold to 2-fold or MR1). Results from this study can be used to develop analytical devices for protein folding and quick recovery of bioactive proteins from inclusion bodies.

P-012

Protease-immobilized microreactor for rapid protein digestion

Masaya Miyazaki, Takeshi Honda, Hiroshi Yamaguchi, Hideaki Maeda

Nanotechnology Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Japan

Proteolysis is the key step for positive sequencing in proteomics analysis integrated with mass spectroscopy. The conventional techniques of in-solution digestion of proteins offer limited sensitivity and time-consuming procedures thus, affecting severely the determination of comprehensive proteomic profiles. To solve this problem, immobilized-enzyme has been widely utilized owing to their advantage of allowing use of higher enzyme concentrations that lead to shorter digestion time. Furthermore, the immobilized-enzyme could be easily isolated and removed from the protein digests prior to MS, therefore, eliminating the influence of fragments from the enzyme on MS results. Several reports have demonstrated the feasibility of protein digestion using enzyme immobilized on various supports such as sol-gel supports, polymer, and membrane. However, preparations of these enzyme-immobilized supports require high-level techniques and multi-step procedures.

We previously developed a facile and inexpensive preparation method for an enzyme-immobilized microreactor. Immobilization of enzymes can be achieved by the formation of an enzyme-polymeric membrane on the inner wall of the microchannel through cross-linking polymerization in a laminar flow [1]. Using this method, we developed a novel microreaction system for optical resolution of racemic amino acids [2]. In the present study, we prepared protease-immobilized microreactors for rapid protein digestion in proteomics analysis. Trypsin, V8 protease, and elastase, which have different pI values, were used. The immobilized-protease showed rapid protein digestion compared to that of in-solution digestion. Furthermore, the stability of enzyme against temperature was enhanced. Details of digestion activity of protease-immobilized microreactor is reported in this paper.

[1] Honda, T., et al., (2006) *Adv. Synth. Catal.*, **348**, 2163-2171.
[2] Honda, T., et al., (2007) *Lab. Chip.*, **7**, 366-372.

P-013

Conformation analysis of prion protein using fluorescence immunoconformational correlation spectroscopy (FiCS)

Hiroshi Sakata[1], Motohiro Horiuchi[2], Masataka Kinjo[1]

[1]*Division of Cellular Life Science, Faculty of Advanced Life Science, Hokkaido University,*
[2]*Laboratory of Prion Diseases, Graduate School of Veterinary Medicine, Hokkaido University, Japan*

Prion diseases are characterized by the conversion of soluble isoform of prion protein (PrPC) to insoluble, disease-causing isoform (PrPSc). However, soluble oligomeric structures are believed to be early stage from the monomeric PrPC to insoluble PrPSc. To detect the oligomerization of PrP, we propose a new immunoconformational assay using fluorescence correlation spectroscopy (FCS) and a series of PrP specific monoclonal antibodies (mAbs). FCS enables direct detection of the translational diffusion (DT) of fluorescent molecule in solution. Dual color fluorescence cross-correlation spectroscopy (FCCS) can detect the coincidence of two molecules labeled with spectrally distinct fluorescent probes. To clarify whether amino acid sequence (epitope) against the mAb is surface-exposed or buried, green fluorescent protein (GFP) fusion PrP (GFP-PrP) and PrP specific mAb labeled with Alexa647 (mAb647) were employed. In vitro oligomerization of GFP-PrP was achieved by using low concentration (<0.01%) of SDS. With increasing concentrations of SDS (from 0.002% to 0.008%), the DT of the fusion protein obtained from FCS became slower. This indicates that the fusion protein became to oligomer or multimer from monomer. In contrast, in between 0.009% and 0.01% SDS the DT became faster but still slower than that of the monomeric fusion protein. FCS gives us other important information; fluorescent intensity of single fluorophor molecule named as usually Counts per Molecule (CPM). The CPM value of GFP-PrP in the presence of 0.009% to 0.01% SDS was about 3 times in comparison with the value of the monomer in the native condition (SDS 0%). FCS indicates that the oligomeric GFP-PrP is trimer form. Using a series of PrP specific mAb647s, we further achieved FCCS measurement to confirm whether the oligomeric GFP-PrP has any conformational changes or not. PrP region of amino acid 219-229 was shown to be cryptic under native conditions and solvent-exposed under oligomeric condition (SDS 0.009%-0.01%). PrP regions of amino acid 56-89 and 143-149 were solvent-exposed in both conditions. Our studies provide a novel tool for analyzing the conformation of oligomeric PrP in solution.

P-014

A novel method for the detection and analysis of protein pockets and cavities using a rolling probe sphere

Jian Yu, **Min Yao**, Isao Tanaka

Graduate School of Life Science, Hokkaido University, Japan

The identification of binding sites from protein structures is helpful as it may provide a key to apprehend proteins of unknown function while their structure has been solved. In general, binding sites of protein are pockets (on protein surface) and cavities (inside protein) which are formed by peptidechain. Therefore, the detection and analysis of pockets and cavities is useful for predicting function of protein and also is a prerequisite for protein-ligand docking and an important step in structure-based drug design.

At present, two kinds of geometric algorithms are widely used for searching binding sites of protein: 1) Grid system and 2) Probe sphere. Based on the analysis of the existing methods, a new method which uses a scroll probe sphere to detect pockets and cavities of protein, is presented here. At first, a 3D gird system is filled with protein atoms and then a probe sphere will roll along the protein surface to form the "probe surface". Pockets and cavities can be found between the two surfaces. A novel function of this method is that pockets with different size can be detected by adjusting the radius of probe sphere. Moreover, in order to suppress noise, two novel parameters Single-Point Flag (SPF) and Protein-Depth Flag (PDF) are developed for more accurate searching results. The result of the test with 53 complex structures containing sixty five ligands randomly downloaded from PDB website showed that our program had a good performance of detecting binding sites. The successful rate of finding binding sites in top three pockets or cavities is 92% and some shapes of them are very similar to the bound ligands. In addition, compared with two other pocket-searching programs, it's indicated that our program runs faster. The time consumption for each case of the 53 is only a few seconds.

After the determination of pockets and cavities, the future plan of our research is to analyze their properties which can be divided into two categories: geometric and chemical property, in order to give rough interaction probabilities of ligands selected from the database.

P-015

Sensitive and unequivocal determination of pKa values of individual histidine residues in proteins using mass spectrometry

Masaru Miyagi[1], Takashi Nakazawa[2]

[1]*Center for Proteomics, Case Western Reserve University,* [2]*Department of Chemistry, Nara Women's University, Japan*

We developed a mass spectrometric method to determine the pKa values of individual histidine residues in proteins. The method utilizes pH-dependent hydrogen-deuterium (H-D) exchange kinetics at the imidazole C_2-position of histidine residues and involves: 1) protein incubation in D_2O solvent at various pH values, 2) protein digestion by proteolytic enzyme(s), and 3) measurement of the mass spectrum of each histidine-containing peptide by LC/ESI-MS. The rate constant of the H-D exchange reaction is obtained from the mass spectrum reflecting the extent of deuterium incorporation. The pKa value is then determined from a plot of the rate constant versus pH, which gives a typical sigmoidal curve. Unambiguous assignment of the pKa values to individual histidine residues can be achieved simultaneously based on the observed molecular mass of the peptide. The pKa values of three of four histidine residues (His12, 105, and 119) in RNase A were successfully determined by this method and were in good agreement with those determined by ^{1}H NMR and hydrogen-tritium (H-T) exchange methods. The method uses sub nanomole quantities of protein, allowing measurement at a much lower concentration than that of 1 mM required for the conventional NMR approach that is currently almost exclusively the method of choice.

P-016

Multicolor detection of posttranslational modifications of proteins on a single 2-DE pattern by quantum dot technology

Masamichi Oh-Ishi, Yoshio Kodera, Tadakazu Maeda

Department of Physics, Kitasato University School of Science, Japan

The number of protein isoforms in the human proteome has known to be much higher than the number of genes in the human genome. This is in large part due to posttranslational modifications (PTMs) of proteins. Knowledge of these PTMs is extremely important because they may altar physical and chemical properties, folding, complex formation, stability, activity, and consequently, function of the proteins.One of the most useful aspects of two-dimensional gel electrophoresis (2-DE) for proteomics is its ability to resolve the modified protein entities into separate spots that can be individually measured. The mass spectrometry methods used to identify the protein in a spot from a 2-D gel are excellent, but have some disadvantages when it comes to determining if a protein is or is not modified. An alternative approach for determining PTM types of a protein spot on a 2-D gel is to stain only the protein with a certain type of PTM with specific dyes such as Pro-Q^{TM} Diamond (phosphoproteins) or Pro-Q^{TM} Emerald (glycoproteins).With quantum dot^{TM} technology, we developed a new proteomic method for simultaneous and multicolor detection of various PTMs of proteins on a single Western or immunoblotting analysis. We applied this method to examine the changes of the PTMs of proteins from various tissues and cells in a diabetes model Otsuka Long-Evans Tokushima Fatty (OLETF) rat. Protein carbonyls, ubiquitination and advanced glycation end products (AGEs), were detected simultaneously on a single blot. A number of proteins were found carbonylated. However, there were few proteins which were simultaneously both carbonylated and ubiquitinated. Our method would provide a means toward clarifying a comprehensive view of abnormal PTMs of proteins on disease proteomics.

P-017

Isolation of C-terminal peptides of proteins by exhaustive amidation followed by proteolytic digestion for sequencing with mass spectrometry

Mariko Nakagawa[1], Minoru Yamaguchi[2], Hiroki Kuyama[3], Chihiro Nakajima[3], Eiji Ando[2], Susumu Tsunasawa[3], Osamu Nishimura[3], Takashi Nakazawa[1]

[1]Department of Chemistry, Nara Women's University, [2]Life Science Laboratory, Shimadzu Corporation, [3]Institute for Protein Research, Osaka University, Japan

The C-terminal sequencing can be useful for identifying proteins, in particular, when the N-terminal amino groups are blocked by post-translational modifications. We have developed a simple method for isolating the C-terminal peptide to be analyzed the sequence by mass spectrometry. The method uses the fact that every peptide fragment except for the C-terminal one in proteolytic digest of a protein could have one free carboxyl group if all the carboxyl groups of a protein have been chemically modified by amidation. We will report the procedures of the method together with the reaction conditions of chemical modification, ion-exchange chromatography to isolate the C-terminal peptide, and MALDI mass spectrometry of the C-terminal peptide thus isolated.

The method consists of the following three steps: (i) exhaustive esterification/amidation of the carboxyl groups in a protein, (ii) proteolytic digestion of the modified protein, and (iii) ion-exchange chromatography to trap all the peptides other than the C-terminal one, which should solely lack the free carboxyl group. We applied the technique to beta-lactoglobulin and BSA, in which all the carboxyl groups were modified with methylamine, added in large excess by using water-soluble carbodiimide (EDC) as a condensation reagent. The C-terminal peptide in tryptic digest was isolated by chromatography using a strong anion-exchange resin (SAX). Various combinations of amines, condensation reagents, and adsorbent for peptides bearing the free carboxyl group were tested. The C-terminal peptide of beta-lactoglobulin was certainly enriched to the extent such that it exhibited the most intense MALDI peak at m/z 1768. The subsequent MALDI-PSD mass spectrum of this peak was in agreement with the sequence: LSFNPTQLE*E*QC*HI** (* and ** denote the side-chain and the C-terminal carboxyl groups, respectively).

Thus, we envisage that this simple method could be used for identifying proteins based on the C-terminal amino acid sequences.

P-018

One-pot incorporation of various functional groups into polypeptide chains by RNA catalysts

Masaki Ohuchi[1], Hiroshi Murakimi[2], Hiroaki Suga[3]

[1]*Department of Advanced Iterdisciplinary Studies, Graduate School of Engineering, The University of Tokyo,* [2]*Reserch Center for Advanced Science and Technology, The University of Tokyo,* [3]*Department of Chemistry and Biotechnology, Graduate School of Engineering, The University of Tokyo, Japan*

Flexizymes are artificial RNA catalysts that facilitate the acylation of tRNAs with highly flexibility to both structure of its substrates and kinds of tRNAs. It recognizes the 3'-end of tRNA (RCCA-3', R = G or A as the discriminator base) as well as a benzylic moiety on the leaving group, and catalyze tRNA aminoacylation. The flexibility of this de novo acylation system enable us to choice wide variety of acid substrates including many of non-natural alpha-amino acids, D-amino acids, N-methyl amino acids, beta-amino acids and alpha-hydroxy acids. Although the flexizyme system provide technical advantages for the versatility to both structures of substrates and kind of tRNAs, the specific acylation of a tRNA by flexizymes could not be carried out under the condition existing multiple kinds of tRNAs because it recognize conserved sequences of tRNAs (CCA sequences). Previously, to apply the flexizyme system for non-natural peptide or protein synthesis, the acylation of tRNAs had to perform in ex situ of the translation apparatus. In order to expand the utility of the flexizyme system, here we report constructions of the in situ generation system of non-standard aminoacyl-tRNAs in a E.coli reconstituted cell-free translation system (PURE system) by coupling the RNase P and catalytic precursor tRNAs that have flexizyme sequences in 5' reader regions. In situ aminoacylation assays indicated that the in cis aminoacylation of precursor tRNAs were effectively conducted while the digested flexizymes had no activity under the condition of the in vitro translation system. Coupling catalytic precursor tRNAs and RNase P described here enable to incorporate various non-standard functional groups into polypeptide chains with high-throughput manner.

P-019

Post-genome analysis of ailkworm (Bombyx mori) and the construction of proteome database

Hideyuki Kajiwara[1], Atsue Imamaki[2], Masatoshi Nakamura[1], Kazuei Mita[1], Chikatada Satoh[3], Yuji Shimizu[3], Michihiko Shimomura[3], Masumi Ishizaka[4]

[1]National Institute of Agrobiological Sciences, [2]Environmental Research Center, [3]Mitsubishi Space Software, [4]National Institute for Agro-Environmental Sciences, Japan

Proteomic analysis of silkworm (*Bombyx mori*, p50) based on the two-dimensional polyacrylamide gel electrophoresis and the mass spectrometry were performed and the proteome database (http://kaiko2ddb.dna.affrc.go.jp/cgi-bin/search_2DDB.cgi) was constructed using Make-2DDB II software (http://br.expasy.org/ch2d/make2ddb/) for the basic research of insect morphogenesis and the use of industrial purpose such as the production of useful substances. The present silkworm proteome database contains the proteomes of the eight major tissues in the silkworm larva and pupa: the middle silkglands, posterior silkglands, midguts, fat bodies, hemolymph, ovaries, testes, and Malpighian tubules. Proteins in each tissue at day three of the fifth instar larva were identified by Sequest and Mascot using amino acid sequence data obtained by the open reading frame analysis of genome sequences. In the case of midgut, more than 60% of the proteins analyzed by the mass spectrometry were identified. These included several kinds of cytoskeleton proteins, ATPases, and chaperonins. Protein profiles by two-dimensional polyacrylamide gel electrophoresis from day one of the fourth instar larva to the adult moth were listed. Protein expression pattern and the identified proteins were compared. It is strongly expected to identify the post-translational modifications on proteins related to the silkworm developments for the next step. We had started the identification of those modifications such as phosphorylation, glycosylation, biotinylation, and S-nitrosylation using Western blotting techniques and mass spectrometry.

P-020

Label-free quantitative proteomics analysis of kidney glomeruli in ADR-induced proteinuric mice

Masatoshi Nukui, Gunvor Alvelius, Karl Tryggvason

Division of Matrix Biology, Department of Medical Biochemistry Biophysics, Karolinska Institutet, Sweden

In many kidney diseases the glomerular filtration barrier is affected, resulting in the leakage of proteins to the primary urine. Proteinuria is characterized by large plasma proteins in the urine and it leads to end-stage renal disease. Proteinuria also is one of the most common indications of glomerular dysfunction. The molecular mechanisms underlying this manifestation, however, are still poorly understood. Evaluating the difference of protein-expression levels between healthy and proteinuric mice glomeruli reveals which proteins are playing important roles in causing proteinuria.
Adriamycin (ADR)-induced nephropathy is a murine experimental model of chronic renal injury. Overt proteinuria was observed 4 days after injection and the leakage of serum albumin reached maximum 7 days after injection. Earliest morphological changes in kidney were observed by electron microscopy as segmental foot process effacement occurred 7 days after injection. Light microcopy showed tubular casts secondary to massive glomerular proteinuria 14 days after injection.
Total protein was extracted from glomeruli at 2 different time points, 0 day and 7 days after injection of ADR. After tryptic digestion, pre-digested Enolase was added to samples as a semi-internal standard. Capillary liquid chromatography of samples was performed with a Waters nano ACQUITY UPLC System equipped with a Waters BEH 130 reverse phase column. Mass spectrometry analysis of samples was performed in triplicate using a Waters Q-Tof Premier. Acquired LC/MS/MS^{E} data sets were analyzed for qualitative protein identification and for relative quantification between 2 sample sets. Although more than 200 proteins were identified in each run, no significant protein expression difference between 2 samples was detected.
The obtained result was due to the dynamic range of protein expression in glomeruli; expression levels of proteins playing important roles to cause proteinuria were much lower than those of house keeping proteins. To overcome this challenging situation, we started utilizing new methods for running samples on mass spectrometer, and results are now under way.

P-021

Protein identification and quantification using a proteome database of liquid chromatography-mass spectrometric data

Hiroshi Kawasaki, Noriaki Arakawa, Hisashi Hirano

Supramolecular Biology, International Graduate School of Arts and Sciences. Yokohama City University, Japan

The peptide identification capability of LC-MS (liquid chromatography-mass spectrometry) enables higher sensitivity measurements of changes in protein expression within a cell. We constructed a proteome database of peptides, which includes amino acid sequences, retention times of peptides and peak hights. LC-MS/MS analysis of the digest was performed on a Hitachi Nanofrontier LD LC-MS system. Peptides in the injected sample solution were trapped in a monolith column (Monolith Trap C18-50-150L) and separated on a reversed-phase column (MonoCap for Fast Flow, 0.05 i.d. x 150 mm) by linear gradient elution with acetonitrile in 1% formic acid at a flow rate of 200 nl/min. Peptides eluted from the column were subject to electrospray ionization and analyzed in a linear ion trap time of flight mass spectrometer. MS/MS analysis was triggered with an information-based acquisition system. Peptide sequences were determined by searching the database (SwissProt) with the Mascot search engine. The retention times of identified peptides were extracted from LC-MS and MS/MS data. Data processing and the extraction of retention times were performed with a utility software package provided by Hitachi. LC-MS or LC-MS/MS (liquid chromatography-mass spectrometry/mass spectrometry) data can be also utilized to establish a relationship between LC (liquid chromatography) retention times and the empirical hydrophobicity of an arbitrary set of standard peptides. We compared proteins expressed in serum and in ovarian cancer cells by clustering peptide peak heights in the database. Peptide clusters revealed a protein expression profile of each sample analyzed. An observed hydrophobicity can be determined for unknown peptides based on their retention times relative to those of the peptide standards. Incorporating this type of LC-MS data into a public proteome database would allow direct comparisons and data exchange between laboratories for large-scale analyses.

P-022

An approach to the quantitative analysis of free thiol levels in human CSF proteins by the method of 2-D gel fluoroimaging with using cyanine dyes and fluorescent gel stain

Tosifusa Toda[1], Megumi Nakamura[1], Hiraku Morisawa[1], Machiko Iwamoto[2], Junpei Ohkata[3], Hajime Sato[3], Hiroshi Tanaka[3], Kazutomi Kanemaru[4]

[1]*Research Team for Molecular Biomarkers, Tokyo Metropolitan Institute of Gerontology,*
[2]*Research Team for Functional Genomics, Tokyo Metropolitan Institute of Gerontology,*
[3]*Bio-Rad Laboratories,* [4] *Tokyo Metropolitan Geriatric Hospital, Japan*

Many neuropathologists have suspected that the relationship between mild cognitive impairment (MCI) and the progression to Alzheimer's disease (AD), however, there has been no direct evidence obtained yet. Recently, Bermejo and his coworkers reported their data showing a decrease in GSH levels and an increase in protein oxidation levels in AD and MCI patients compared to age-matched control subjects (Free Radic. Res, 42(2), 162-170, 2008). The data suggests that oxidative stress might represent a signal of early stage of AD pathology. The diagnostic value of cerebrospinal fluid (CSF) biomarkers such as phosphorylated tau protein and beta amyloids in advanced AD patients has been already realized, however, the diagnostic value of measurement of protein redox level in early stage of AD patients has not yet been qualified. Thiol-specific maleimide derivatives of cyanine dyes, that have been already widely used for saturation labeling all thiols and disulfides in 2D-DIGE), are also useful to analyze protein redox level by fluoroimaging. Thus we tried to establish a convenient protocol for quantitative analysis of the free thiol level of proteins on a 2-DE in combination of the cyanine-dye labeling and fluorescent gel staining. In this paper, we report the protocol and the results obtained in our recent studies on quantitative analysis of free thiol level of proteins in human CSF.

P-023

Comprehensive analysis of functional proteins related to the neuronal differentiation in PC12 cells induced by NGF stimulation using iTRAQ method and bioinformatics with GO analysis

Daiki Kobayashi[1], Takashi Morikawa[1], jiro Kumagai[2], Anthony Wilson[1], Masayo Wilson[1], Megumi Nagayama[1], Takeru Nanbu[3], Norie Araki[1]

[1]*Department of Tumor Genetics and Biology, Graduate School of Medicine, Kumamoto University,*
[2]*Core Laboratory for Medical Research and Education, Medical School, Kumamoto University,*
[3]*Department of Pharmacy, Kumamoto University Hospital, Japan*

Nerve growth factor (NGF)-induced neurite outgrowth of rat pheochromocytoma PC12 cells serves as a representative model systems for studying neuronal differentiation processes. Despite of the extensive biological research, proteomic study of PC12 cells has been limited so far. To get a new set of functional protein information related to the neuronal differentiation processes, we identified the proteins that differentially expressed in PC12 cells before and after treatment of NGF, by using isobaric tagging for relative and absolute quantitation (iTRAQ) coupled with high-sensitive nano-LC-MALDI-MS/MS and nano-LC-ESI-MS/MS systems. Out of about 1500 proteins semi-quantitatively identified from the combined data by both of LC-MALDI- and LC-ESI-MS/MS analysis, 72 were differentially expressed in response to NGF stimulation in PC12 cells; 39 proteins were up-regulated and 33 were down-regulated, and they include several novel proteins in PC12 cells. Gene ontology (GO) analysis revealed that up-regulated proteins included the molecules involved in cell morphogenesis, response to stimulus and biological quality, as well as cell differentiation. We comfirmed the expression levels of these proteins related to cell morphogenesis or cell survival by Western blotting. Four proteins, TCTP, NRAGE, Prothymosin alpha and PAI-RBP1 uniquely identified in this study, were further analyzed to validate their functions on neuronal differentiation by using specific siRNA technique. Suppression of these proteins caused the morphological changes or apoptosis of PC12 cells. These results suggest that the NGF-responsinve proteins identified in this study are essential for neuronal differentiation, and iTRAQ and GO analysis are useful for the comprehensive functional proteome analysis to understand cellular biological mechanisms.

P-024

GH transgenic salmon proteomics

Youichi Kurata[1], Tsukasa Mori[2], Yuko Yamanaka[1], Hisashi Hirano[1]

[1]*International Graduate School of Arts and Sciences, Yokohama City University,* [2]*College of Bioresource Sciences ,Nihon University, Japan*

Growth hormone (GH) transgenic amago salmon (Oncorhynchus masou) was generated with a construct containing the sockeye salmon GH1 gene fused to the metallothionein-B (MT-B) promoter from the same species. The growth of transgenic salmon was enhanced and the weight of them increased four or five times compared to the non-transgenic salmon. This drastic growth enhancement by GH transgene is well known in fish species (especially Salmonidae) compared to mammals, however, such a fish shows morphological abnormalities and physiological disorders as reported in other GH transgenic animals. GH is known to have many acute effects, but currently there are no data describing the chronic effects of over-expression of GH on various pituitary proteins in GH transgenic fish. Since pituitary is a main organ for synthesis and secretion of GH, regulation of growth, reproduction, homeostasis and pituitary protein expression, it plays important roles in various physiological functions and growth performance of the transgenic and non-transgenic salmon. To examine GH effects on the pituitary, we cultured pituitaries extracted from GH transgenic salmon. Proteins were extracted from these cultured pituitaries and analyzed using isobaric tags for relative and absolute quantitation (iTRAQ) method combined with MALDI-TOF/TOF mass spectrometer. In iTRAQ analysis, 2,345 pituitary proteins (45,305 spectra included) were identified and about 10 % of them were differentially expressed in pituitaries from the GH transgenic salmon compared to the non-transgenic ones. These proteins were related to growth, metabolism, and reproduction. Dynamics of these protein expressions in the GH transgenic salmon were compared to those in the non-transgenic salmon using hierarchical and functional annotation clustering analysis. The protein expression patterns observed in GH transgenic salmon were different from those in non-transgenic salmon injected high concentration of GH, suggesting that this difference might be caused by difference of dynamics of these protein expressions between transgenic and non-transgenic ones.

P-025

Differential expression profile of protein tyrosine kinases in the mouse crypt cells of the oxazolone-induced ulcerative colitis model

Shingo Semba, Yanju Ma, Tsuyoshi Katoh, Takanobu Taniguchi

Department of Biochemistry, Asahikawa Medical College, Japan

BACKGROUND AND AIMS Ulcerative colitis (UC), which is one of the inflammatory bowel diseases, is chronic inflammatory disorders of the gastrointestinal tract, and their pathogeneses are unclear. Recent studies suggest that disruption of immune systems in the intestinal mucosa is involved in the pathogeneses of UC. It is believed that protein tyrosine kinases (PTKs) have important roles in the activation and maturation of immune system, thus we assume that the expression level of PTKs may change in the intestinal mucosa of UC patients. To verify this hypothesis, we investigated PTKs expression level using unique monoclonal antibody (termed as YK34) that detected a variety of PTKs.

METHODS We have employed oxazolone-induced mouse model that was accepted as a T helper-2 type model. Balb/c mice were given 150 uL of 1% oxazolone from the anus through catheters and were sacrificed 2 days after the administration. The crypts of intestinal mucosa were isolated from the distal colon. The protein extracts were prepared from the crypts and were subjected to Western blot analysis using YK34 that was raised against highly conserved region (subdomain VIB) of PTKs.

RESULTS The administration of oxazolone induced severe inflammation in the distal colon at day 2. The mortality of the oxazolone-treated mice by the end of day 2 was approximately 50%. Western blot analysis following 2D-PAGE revealed that several proteins were recognized with YK34. The intensities of at least 4 immuno-reactive spots were dramatically increased by the induction of colitis.

CONCLUSIONS Our results have suggested that protein tyrosine kinases in the intestinal mucosal cells may be involved in the pathogenesis of ulcerative colitis.

P-026

EurocarbDB: A database and software platform for glycoinformatics

Kai Maas[1], **Hildegard Geyer**[1], Rudolf Geyer[1], Alessio Ceroni[2], Stuart Haslam[2], Rene Ranzinger[3], Claus-Wilhelm von der Lieth[3], Matthew Campbell[4], Pauline Rudd[4], Thomas Luetteke[5], Bas Leeflang[5], Magnus Lundborg[6], Goran Widmalm[6], Matt Harrison[7], Kim Henrick[7]

[1]Institute of Biochemistry, Faculty of Medicine, Justus-Liebig-University of Giessen, [2]Imperial College of Science, Technology and Medicine, London, [3]German Cancer Research Center, Heidelberg, [4]National Institute for Bioprocessing Research and Training, University College Dublin, [5]Bijvoet Center, Utrecht University, [6]University of Stockholm, Sweden

EurocarbDB is a European Union-funded initiative that seeks to address the lack of sophisticated, freely accessible informatics tools for the rapidly expanding field of glycobiology. EurocarbDB comprises a publically curated, open-access database of carbohydrate structures and primary research data, accessed via a web-based browse/search/contribution interface. The database component is complemented by a suite of associated glyco-informatics tools, designed to aid in the elucidation and submission of glycans structures when used in conjunction with contemporary carbohydrate research workflows. Mass spectrometry is the main analytical technique currently used for rapid and reliable glycan analysis. Determination of glycan structures from analysis of MS data is a major bottleneck in high-throughput glycomics, and robust solutions to this problem are of critical importance. In the EuroCarbDB initiative several software tools were developed to assist the rapid interpretation of MS data: The *Glyco-Peakfinder* web application is designed for de novo composition analysis of mass signals of glycans. The tool assigns all types of fragmentations including monosaccharide cross-ring cleavages and multiply charged ions. To provide access to known carbohydrate structures a 'composition search' in an open access database can be performed. The *GlycanBuilder* has been developed as a fast and easy to use tool for drawing and displaying glycans in a chosen symbolic notation. Both tools have been integrated into the *GlycoWorkbench* software suite, a collection of resources for semi-automated profiling and sequencing of glycans from MS data. Once the possible structure candidates for a mass signal have been identified with *Glyco-Peakfinder* and/or drawn with the *GlycanBuilder*, the corresponding theoretical lists of fragment masses can be matched against a list of peaks derived from a MS spectrum to determine the most plausible structure. This collection of software tools offers complete support for routine analysis of MS data and is freely available at the URL: www.eurocarbdb.org/applications/ms-tools. In addition, HPLC and NMR components will be implemented in EurocarbDB soon.

P-027

Proteomics-based protein profiles of unfertilized egg, tadpole larva and neural complex in *Ciona intestinalis* for an integrated protein database CIPRO (*Ciona intestinalis* Protein database)

Ayako Nakajima, Mia Nakachi, Mamoru Nomura, Kazuo Inaba

Shimoda Marine Research Center, University of Tsukuba, Japan

CIPRO is an integrated protein database of an ascidian, *Ciona intestinalis* and is aimed for utilizing various levels of information from genome to individual. It includes information on genome, gene expression profiles, MS/MS sequence tags, 2D-PAGE images, intracellular protein localization, 3D images of the embryos and transgenic resources.

Ascidians are primitive chordates providing excellent experimental model systems in a variety of fields, in particular developmental biology. They develop in a typical mosaic way and hatch out into tadpole larvae, which metamorphose into adults through juveniles. The tadpole larvae have a primitive chordate body plan and share the fundamental features with vertebrates. However, they are composed of only ~2,600 cells and we can analyze them at the single cell level. The neural complex of mature adults is considered as an ancestral form of the vertebrate CNS (central nervous system) and serves as a model for neuroendocrinological studies.

C. intestinalis is a cosmopolitan species and is becoming prominence as a model organism. The genome contains basic set of vertebrate genes but the constitution is very compact with the size of ~160 Mb and only ~16,000 protein-coding genes. Comprehensive EST analysis has been performed using cDNA libraries from the embryos in principal developmental stages and from the tissues of mature adults. Gene expression profiles were also examined extensively using whole mount in situ hybridization and DNA microarrays. Although the information of genome sequences allowed proteomic analysis, there have been few studies on protein expression profiles.

We are preparing data sets composed of 2D-PAGE images and the spot identifications through peptide mass fingerprinting. In CIPRO, the protein profiles will be integrated with the available information on genome and gene expression profiles. As a part of the CIPRO resources, we report here the proteomics-based protein profiles of unfertilized egg, tadpole larva and neural complex. (Supported by JST-BIRD. The first three authors equally contributed to this work.)

P-028

CIPRO: an integrated protein database of the ascidian Ciona intestinalis

Toshinori Endo[1], Keisuke Ueno[1], Koki Yonezawa[1], Katsuhiko Mineta[1], Koji Hotta[2], Lixy Yamada[3], Hisaaki Taniguchi[3], Michio Ogasawara[4], Yutaka Satou[5], Mamoru Nomura[6], Ayako Nakajima[6], Mia Nakachi[6], Yasunori Sasakura[6], Kazuo Inaba[6]

[1]Information Science and Technology, Hokkaido University, [2]Biosciences and Informatics, Keio University, [3]Institute of Enzyme Research, The University of Tokushima, [4]Faculty of Science, Chiba University, [5]Graduate School of Sceince, Kyoto University, [6]Shimoda Marine Research Center, Tsukuba University, Japan

CIPRO is developed to provide widespread information of the proteins of the ascidian *Ciona intestinalis* which belong to the same phylum with vertebrates. Since *C. intestinalis* provides advantages especially experimental design, including the traceable developing stages and a concise resource of its genome, gene and protein expressions. As a bioinformatics resource, CIPRO provides the basic information for the *Ciona* proteome, including amino acid sequences for the CIPRO gene model, predicted isoelectric points and molecular mass as well as virtual 2D-electrophoresis image, three-demensional structure models, subcellular-localization, homology search results. It serves a Blast homology search service and virtual PMF fingerprints for input protein sequences. It also provides the experimental data, including the slice images of developing stages from a single celled egg to the hatching stage of larva, the corresponding EST levels, 2D-gel spot images, peptide mass fingerprints (PMF). Transgenic resources is planed be supplied in the near future. The resource intended to serve as the start point to the biological and biomedical researches.

P-030

Single molecular tracking of cholera toxin subunit B on GM1-incorporated in self-spreading lipid bilayer

Toshinori Motegi, Baku Takimoto, Hideki Nabika, Kei Murakoshi

Department of Chemistry, Faculty of Science, Hokkaido University, Japan

Molecular manipulation on a lipid bilayer is an effective approach to manipulate, segregate, and purify bio-molecules in their native environment. Most reported approaches are based on an electrophoresis, which imposes severe restriction that non-charged molecules cannot be manipulated. To overcome this limitation, self-spreading lipid bilayer has recently been attracted a growing interest as novel molecular transport ability. The self-spreading phenomenon of lipid bilayer is thermodynamically driven collective molecular flow. Thus, the self-spreading lipid bilayer can transport non-charged molecules in any direction in the absence of outer perturbation. We have discovered a molecular filtering phenomenon at the self-spreading lipid bilayer passing through a periodic array of metallic nano-gates. A formation of a highly localized compressed phase at the gates acts as a chemical potential barrier for the filtering of the target molecules in the bilayer. The chemical potential barrier at the gates prevents the diffusion of the target molecules through the gate. The phenomenon has been expected to be applied for the construction of novel molecular filtering system.

In the present study, we extended our system to bio-molecules for the verification of advantage of the method. Direct incorporation of membrane protein or peptides frequently induces undesirable interactions between the incorporated molecule and the solid substrate. To avoid this situation, we started our experiment with ligand-receptor conjugate on the lipid bilayer. As the first step, binding characteristics of cholera toxin subunit B (CTB) to ganglioside GM1 (GM1) incorporated in the self-spreading lipid bilayer was investigated by a single molecule tracking experiment. The diffusivity of CTB-GM1 conjugates was found to be changed dynamically during the observation. Time-dependent change in the diffusivity was critically dependent on a concentration of both CTB and GM1. The present results demonstrated direct information on a dynamic change in the association and/or coordination of CTB with GM1. As the next step, we have investigated the effect of nano-gates for the CTB diffusion in order to establish a novel molecular manipulation system.

P-031

Dynamic adsorption and insertion behavior of MSI-78 on a self-spreading lipid bilayer

Hideki Nabika, Masahiro Oowada, Baku Takimoto, Takao Nomura, Yoshiro Chuman, Kazuyasu Sakaguchi, Kei Murakoshi

Division of Chemistry, Graduate School of Science, Hokkaido University, Japan

Artificial lipid bilayers have long been used by many researchers as a model system for a cell membrane. Recently, a spontaneous growth of the lipid bilayer from a lipid aggregate, which is called as a self-spreading, have also attracted growing interest for molecular transportation, sorting, and separation. One of superior advantages of the self-spreading nature is the fact that the molecular transportation and manipulation can be done without any external bias, such as an electric field in an electrophoresis. In spite of its fruitful nature, previous reports were limited to manipulate only dye-labeled lipids in the self-spreading lipid bilayer. There was no effort toward the use of bio-molecules. To prove the ability of manipulating bio-molecules in the self-spreading bilayer, dynamic adsorption and insertion behavior of antimicrobial peptide MSI-78 onto the self-spreading lipid bilayer was investigated in the present study.

A small quantity of DLPC chloroform solution was dropped on a clean cover glass. After drying the chloroform, the substrate was immersed in phosphate buffer. Just after the immersion, a single bilayer was evolved from the DLPC droplet on the substrate via the self-spreading. During the spreading, phosphate buffer containing ATR-labeled MSI (MSI-ATR) was added to be the final MSI-ATR concentration of 1 μM. Just after the MSI-ATR addition, adsorption of MSI-ATR onto the self spreading lipid bilayer was observed on the fluorescence microscopy. The adsorption saturated after ca. 1000 sec. During observation, MSI-ATR adsorption occasionally induced linear domain formation on the self-spreading lipid bilayer. Also, a one-dimensional (linear) diffusion along the linear domain was observed at the single molecule tracking observation. The present study is the first result on the incorporation of bio-molecule in the self-spreading lipid bilayer. Further investigation will clarify the key roles on the dynamics and manipulation toward novel molecular separation or filtering systems.

P-033

A viral mechanism for dysregulation of post-translational modification in Kaposi's sarcoma-associated herpesvirus latency

Masahiro Fujimuro[1,2], Hideyoshi Yokosawa[1,3]

[1]Biochem. Grad. School of Pharm., Hokkaido University, [2]Mol. Cell Biol., Interdisciplinary Grad. School of Medicine and Engineering, Univ. of Yamanashi, [3]Research Resources Center, Brain Science Institute, RIKEN, Japan

Kaposi's sarcoma associated herpesvirus (KSHV), also known as human herpesvirus 8, is associated with Kaposi's sarcoma (KS) and AIDS-related primary effusion lymphoma (PEL). The latency-associated nuclear antigen (LANA) of KSHV is required for maintenance of KSHV episomal DNA and is believed to play an important role in KSHV latency and tumorigenesis. LANA tethers episomal genome to host chromosomes to ensure viral DNA replication. In addition, LANA interacts with pRb and p53 to promote proliferation and to protect against apoptosis.

We reported that beta-catenin is overexpressed in PEL and in KS. LANA binds the beta-catenin negative regulator GSK-3beta and causes nuclear accumulation of GSK-3beta. This activity correlates with stabilization of beta-catenin. LANA C-terminus contains sequences similar to the GSK-3beta-binding domain of Axin. Disruption of this region resulted in a mutant LANA that failed to re-localize GSK-3beta and stabilize beta-catenin. LANA N-terminus is phosphorylated by the GSK-3beta with a MAPK and CKI functioned as priming kinases. The loss of GSK-3beta phosphorylation of this N-terminal domain correlated with loss of GSK-3beta interaction. Although LANA-associated GSK-3beta actively phosphorylated LANA, GSK-3beta coprecipitated with LANA was unable to phosphorylate an exogenous peptide substrate.

Furthermore, we have found viral dysregulation of post-translational modification for cellular oncoprotein. GSK-3beta phosphorylated the eleven lysine-rich leukemia (ELL) nuclear oncoprotein which functions as anti-apoptotic. GSK-mediated phosphorylation induced the degradation of ELL in a ubiquitin dependent manner. Interestingly, LANA abolished GSK-induced ELL degradation and LANA additionally increased SUMO1 modification of ELL. This LANA-mediated SUMOylation is dependent on LANA binding to ELL. LANA induced the ELL stabilization and SUMOylation that can effect its subcellular localization and activities. ELL-mediated anti-apoptotic activity may play an important role in KSHV tumorigenesis. Further studies to elucidate the functional significance of viral dysregulation of post-translational modification for ELL are under way.

P-034

Large-scale protein phosphorylation profiling by high performance phosphoproteomics based on hydroxy acid-modified metal oxide chromatography (HAMMOC)

Yasushi Ishihama, Naoyuki Sugiyama, Yutaka Kyono, Koshi Imami, Takeshi Masuda, Masaru Tomita

Institute for Advanced Biosciences, Keio University, Japan

Protein phosphorylation is a critical regulatory step in signaling networks and is arguably the most widespread protein modification, affecting almost all basic cellular processes in various organisms. Recent advances in mass spectrometry-based technologies accompanied with phosphopeptide enrichment methods paved the way for high-throughput, large-scale in vivo phosphorylation dynamics. Recently we developed highly specific phosphopeptide enrichment method using hydroxy acid-modified metal oxide chromatography (HAMMOC), where the chemo-affinity of metal oxides to phosphopeptides is enhanced by the addition of aliphatic hydroxy acids [1,2]. This HAMMOC/nanoLC-MS approach allows the identification of more than one thousand phosphorylation sites per single LC-MS run from whole cell lysates without any activation stimulus. Here we will present our recent advances in the HAMMOC approach as well as the application to the phosphorylation profiling of various organisms such as plants [3] and human cancer cells. For Arabidopsis phosphoproteome, we successfully identified more than 3400 phosphorylation sites including 140 tyrosine phosphorylation while no known pY kinase is coded in Arabidopsis genome. In addition, this HAMMOC/nanoLC-MS approach in combination with stable isotope labeling enables to quantify the proteome-wide phosphorylation change induced by stimuli such as growth factors and kinase inhibitor drugs. For instance, we examined human cancer cells treated with an EGFR inhibitor and found that the phosphorylation status of proteins belonging to EGFR pathway was down-regulated by the drug and most of them were not reported as phosphorylated proteins. REFERENCES[1] N. Sugiyama, T. Masuda, K. Shinoda, A. Nakamura, M. Tomita, Y. Ishihama, Mol. Cell. Proteomics, 6 (2007) 1103.[2] J. Rappsilber, M. Mann, Y. Ishihama, Nat. Protoc., 2 (2007) 1896.[3] N. Sugiyama, H. Nakagami, K. Mochida, A. Daudi, M. Tomita, K. Shirasu, Y. Ishihama, Mol. Syst. Biol., 4 (2008) 193.

P-035

Enrichment and analysis of phosphorylated proteins in duchenne dystrophy cells

Roza M. Kamp, Jie Guo-Thuermann, Kunigunde Stephani-Kosin

Department of Biotechnology, Faculty of Life Sciences, University of Applied Sciences Berlin, Germany

Proteins and nucleic acids are the most important biomolecules in the cell. They are involved in many cellular processes, such as signal transduction in all living organisms. Proteins are synthesized on the ribosomes and usually, especially in the eukaryotes, modified after the translation step. A posttranslational modification can assign a protein so it can signalize for example whether an interaction with other proteins is possible or a replacement has to take place. Misled or misplaced signals are a reason for several diseases like cancer, Alzheimer disease, Parkinson or disorders in the embryonic development. Thus, the identification of protein modification, e.g. phosphorylation, is essential to monitor the progress of such diseases. Due to the fact that important steps in cell signalling are regulated by phosphorylation, a comprehensive study including the characterization of phosphorylation-driven signal transduction allows the identification of new target proteins for clinical research. To analyze the low abundant phosphorylated proteins, their concentration usually is lower than 10%, the complexity of all cellular proteins has to be reduced. Routinely, affinity chromatography is applied to enrich phosphorylated proteins. For the enrichment of phosphorylated proteins not only IMAC, but also other methods are available. Affinity chromatography using antibodies is very expensive and only a very limited number of proteins can be separated by this method. The limitations of the chemical enrichment using beta-elimination is that serine reacts faster than phosphorylated threonine and phosphorylated tyrosine does not react with chemicals, such as hydroxide.The advantage of the IMAC-method for the enrichment of phosphorylated proteins is the selectivity of this method. Cross reactions with acidic proteins and free phosphate groups in biological samples can cause contamination of the purified proteins.Because the specifity of commercial IMAC-materials is very limited, a new adsorber for the enrichment of phosphorylated proteins was developed. The phosphoproteins are bound non covalent to the adsorber, which is a mixture of different metal oxide materials, with different metals and behaviours. The proteins are eluted using two different buffer systems. This method allows very specific enrichment of phosphoproteins with high recovery and reproducibility. This approach is applicable for the characterization of whole phosphoproteomes in tissues, organelles and cell cultures.The separation conditions were optimized by adjusting buffer components, pH and concentration of salts and acidic amino acids as well as by modifying the capacity of the adsorber using a complex protein mixture of yeast, rat, muscle and human (HeLa cells) protein extracts. Additionally it is important to protect the phoshoproteins using phosphatase inhibitors and also protease inhibitors because phosphoproteins are unstable and the phosphorylation reaction is reversible. In this way a risk of alteration during the purification processes can be avoided.For the elution of phosphoproteins and for a high recovery two different buffers in two steps (different composition and different pH) are necessary. Otherwise some proteins do not elute from the column and get lost. The two eluted fractions contain partly the same and partly different proteins giving evidence that both buffer systems are necessary to release efficiently the bound proteins. The method for phosphoprotein enrichment and the differential proteome analysis can be applied in basic and medical research to analyze metabolic disorders in all known diseases. We are interested in a detailed research of proteome and phosphoproteome of human skeletal muscle cells with focus on the Duchenne muscular dystrophy (DMD). Metal affinity chromatography was applied for proteome analysis in Duchenne muscle dystrophy cells. The results show differences in the intensity of some proteins, especially of phosphoproteins. It is possible, that these proteins are involved in the signalling pathway in the cell.

P-036

N^{α}-terminal acetylation of ribosomal proteins of *Saccharomyces cerevisiae* and its function.

Masahiro Kamita[1], Roza Maria Kamp[2], Hisashi Hirano[1]

[1]*International Graduate School of Arts and Sciences,Yokohama City University*
[2]*Life Sciences and Technology, University of Applied Sciences, Japan*

Co- and post-translational modifications are essential for many proteins to function. However, the relations between co- and post-translational modifications and function of proteins have not been fully understood. N-Terminal acetylation (N-acetylation) is one of the most common modifications in eukaryote. In yeast, N-acetylation catalyzes with three N^{α}-acetyltransferases called NatA, NatB and NatC. The α-amino group of many proteins is modified with acetyl group from acetyl-CoA by these enzymes. This modification occurs co-translationally on ~50% eukaryotic cytoplasmic proteins. N-Acetylation markedly affects the biological function of proteins. For example, N-acetylation has the effect on the biological activity of the enzymes, protein affinity, heat-stability of proteins and protein turnover mediated by the ubiquitin-dependent degradation system. Many ribosomal proteins are known as being co-translationally modified with acetyl group, but importance of the modification for ribosome functions remains to be resolved. In the present study, two-dimensional difference gel electrophoresis (2-D DIGE) was used to determine the state of N-acetylation of 80S yeast ribosomal proteins from the normal and the N^{α}-acetyltransferase deletion mutants (NatA, NatB and NatC). Previously, it was reported that 23 ribosomal proteins were N-acetylated with NatA, and 2 ribosomal proteins with NatB. Besides these proteins, we found that L5, L13 and L23 ribosomal proteins were acetylated with NatA, and S10 and L16 ribosomal proteins were acetylated with NatB in the present study. We compared poly (U)-dependent poly (Phe) synthesis activity between the normal strain and the N^{α}-acetyltransferase deletion mutants to evaluate the ribosome function. The activity in NatA and NatB deletion mutants was significantly lower than that was the normal strain, suggesting that N-acetylation plays important role in ribosomal functions.

P-037

Posttranslational modifications of the prometastatic protein S100A4

Mads H. Haugen[1], Kjersti Flatmark[1], Svein-Ole Mikalsen[2], Gunhild M. Malandsmo[1]

[1]Department of Tumor Biology and [2]Department of Cancer Prevention, Institute for Cancer Research, Norwegian Radium Hospital, Rikshospitalet University Hospital, Oslo, Norway

S100A4 is a small acidic protein belonging to the S100-family of calcium binding proteins. Elevated levels have previously been correlated with higher metastatic potential in several type of cancer. S100A4 has been linked to multiple cellular events, and has been identified extracellularly, in the cytoplasm and in the nucleus of tumor cells; however, the biological implications of the different subcellular locations are unknown. Associations between a variety of posttranslational protein modifications and altered biological functions of proteins are becoming increasingly evident. Identification and characterization of posttranslationally modified S100A4 variants could thus contribute to elucidating the mechanisms for the many cellular functions that have been reported for this protein, and might eventually lead to identification of novel drugable targets. Using in-house produced antibodies we have immunoprecipitated S100A4 from protein lysates from colorectal carcinoma tumor samples and cell lines. When resolved by 2-dimensional PAGE and visualized by western immunoblotting using another in-house produced antibody, S100A4 showed a characteristic pattern of several horizontally aligned spots, suggesting the presence of at least three charge variants. These charge variants were verified to be S100A4 by mass spectrometry, and this strongly proposes the existence of posttranslationally modified forms of S100A4. Almost identical patterns were observed in samples from different tissues and subcellular compartments, suggesting that the occurrence of the described charge variants is a universal phenomenon. Further investigation will focus on describing the possible nature of these posttranslational modifications and their location in the amino acid sequence using modification specific enzymes and ion trap LC-MS/MS. Potential quantitative differences of the modified forms in colon cancer cell lines and tumor tissues will be explored using DIGE/western immunoblot, and the results of these ongoing studies will be presented.

P-038

Post-translational modification of Chk2 by SUMO, a ubiquitin-like protein

Hidetaka Hosono, Hideyoshi Yokosawa

Faculty of Pharmaceutical Sciences, Hokkaido University, Japan

Post-translational modification plays a crucial role in activation/inactivation of proteins. It has been shown that protein modifications by ubiquitin and ubiquitin-like proteins regulate many cellular events including cell cycle, signal transduction, apoptosis, and autophagy. SUMO (small ubiquitin-related modifier), one of the ubiquitin-like proteins, is conjugated to various proteins (sumoylation) by sequential actions of E1, E2 and E3: SUMO is first activated by E1 (an Aos1-Uba2 hetero-dimer) in an ATP-dependent manner, transferred to E2 (Ubc9), and finally linked to the Lys residue of the target protein, promoted by E3. In this reaction, the target protein is recognized by the E3 and subjected to sumoylation. In the present study, we performed yeast two-hybrid screening using the N-terminal half of PIAS1, a member of the PIAS family functioning as an E3, as bait and identified Chk2 (checkpoint kinase 2) as a novel PIAS1-interacting protein. Chk2 is a key mediator of the DNA damage checkpoint. When DNA double-strand break (DSB) occurs, Chk2 is phosphorylated by ATM and the phospholylated Chk2 activates p53, PML, E2F and so on to regulate cell cycle and apoptosis. Our over-expression and immunoprecipitation analyses revealed that Chk2 is a PIAS1-interacting protein and sumoylated by PIAS1 in a RING finger-dependent manner. It has been reported that Chk2 binds to chromatin and is released when activated by DSB. We found that sumoylated Chk2 binds to chromatin more tightly than unmodified Chk2 does and that Chk2-SUMO fusion protein also binds to chromatin more tightly than the original Chk2 does. In addition, over-expression of PIAS1 enhanced accumulation of Chk2 to chromatin in a RING finger-dependent manner. Interestingly, adriamycin (Adr) treatment led de-sumoylation of Chk2. As efficient activation of downstream effectors needs the release of Chk2 from chromatin, it can be inferred that de-sumoylation of Chk2, promoted by Adr treatment, contributes its release from chromatin and the subsequent efficient activation of the effectors. Thus, we propose that PIAS1 enhances accumulation of Chk2 to chromatin through promoting Chk2 sumoylation.

P-040

Conformational change of Arg123 in chloride uptake of light-driven chloride pump Halorhodopsin

Megumi Kubo[1], Takashi Kikukawa[2], Seiji Miyauchi[3], Masakatsu Kamiya[1], Tomoyasu Aizawa[1], Keiichi Kawano[4], Naoki Kamo[1], Makoto Demura[1]

[1]Faculty of Advanced Life Science, Hokkaido University, [2]Creative Research Initiative Sosei, Hokkaido University, [3]Faculty of Pharmaceutical Science, Matsuyama University, [4]Graduate School of Science, Hokkaido University, Japan

Halorhodopsin (HR) act as a light-driven chloride pump which transports a chloride ion from extracellular (EC) to cytoplasmic (CP) space during a photocycle reaction including some photo-intermediates initiated by illumination. To understand the chloride uptake mechanisms, we focused a basic residue Arg123 of HR from *Natronomonas pharaonis* (NpHR), which is the only basic residue located in the EC half ion channel. By the measurements of the visible absorption spectra in the dark and the light-induced membrane potential, it was showed that the chloride binding and transport ability of NpHR completely disappeared by substitution of arginine by glutamine. From the flashphotolysis analysis, the photocycle of R123Q differed from that of wildtype NpHR completely. Contrary, the response of R123H mutant depended on pH. These facts imply that the positive charge at 123-th position is essential for chloride-binding in the ground state and for the chloride uptake under illumination. On the basis of the structural studies of anion-binding archaeal rhodopsins and the previous reports of the light-driven proton pump bacteriorhodopsin (BR)-Arg82 mutant proteins, the effects of the positive charge and the conformational change of Arg123 side chain as well as the chloride-pumping mechanism was discussed. In this study, we proposed the hydrogen-bonding network model in the EC channel of NpHR, depending on the binding of chloride. The hypothesis is appropriate for describing the uptake and the high-affinity binding of chloride.

P-041

Functional expression of NMDA subtype glutamate receptor by a novel bi-cistronic baculovirus expressoin vector

Tzong-Yuan Wu

Department of Bioscience Technology, Chung Yuan Christian University, Taiwan

N-Methyl-D-aspartate (NMDA) receptors are a subclass of excitatory, ionotropic glutamate neurotransmitter receptors. NMDA receptors are critical mediators of excitatory neurotransmission in the brain, being pivotal for long term potentiation. They are also important as a therapeutic target post-ischemia. NMDA receptor channels are highly permeable to calcium ions, and thus overactivation leads to excitotoxic neuronal cell death. Molecular biology studies had identified the Seven genes encode NMDA receptor subunits NR1, NR2A-NR2D, and NR3A-NR3B. Functional NMDA receptors are formed from the co-assembly of the obligatory NR1 glycine-binding subunit with NR2 and/or NR3 subunits. However, the quaternary structure of NMDA receptors is still not yet established. To functional express these important heteromeric NMDA receptors, a polycistronic eucaryotic expression system is required. We had identified the RhPV IRES (derived from Rhopalosiphum padi virus) and PnV539 IRES (derived from Perina nuda Picorna-like virus) can functional well in baculovirus infected sf21 cells. In this report, we employed these novel baculovirus expression vectors to express NMDA receptors in Sf21 insect cells. Western blot and immunostaining demonstrated the expression of NMDA receptors on plasmamembrane. Calcium image analysis indicated the function of this recombinant NMDA receptor is responsible to NMDA but not to AMPA. These studies will facilitate the protein structure determination of NMDA receptor.

P-042

Protein-free selective retrieval of crosslinked peptides for mass spectrometric analysis of protein complexes

Ruth Hogue Angeletti, Funing Yan, Fa-Yun Che, Dmitrij Rykunov, Carlos Madrid, Edward Nieves, Louis M. Weiss, Andras Fiser

Biodefense Proteomics Research Center, Albert Einstein College of Medicine, USA

Identification of the protein-protein interactions within multi-protein complexes is crucial to understanding biological mechanisms. Through cross-linking, the conformation of the proteins and the contact site of the protein-protein complexes can be better understood. However, data analysis is complicated by the low abundance of the cross-linked peptides within the peptide population of a standard digest. Selective retrieval improves the signal of crosslinked peptides by decreasing irrelevant, non-crosslinked peptides. Use of protein-free selective retrieval eliminates the contamination that often results from avidin-biotin based retrieval systems. In this work, functional, photocleavable cross-linkers were designed, synthesized, and their structure and physical properties validated by mass spectrometry and NMR. We initially characterized these crosslinkers using standard peptides. Following this characterization, human serum albumin (HSA) was chosen as the model protein to establish a method of selective retrieval of crosslinked peptides. Crosslinked protein was digested with trypsin. Cross-linked peptides were captured by beads via a linker. This was followed by extensive washing, after which crosslinked peptides were released from the beads by photo-irradiation with a 355 nm laser. Using nano-LC-MS/MS, peptides were identified that tagged surface Lysine residues, and demonstrated both intramolecular and intermolecular crosslinks. Unique affinity separation techniques coupled with crosslinking minimize background contamination and improve MS signal/noise and sensitivity in mass spectrometry. (Supported by NIH/NIAID contract HHSN266200400054C)

P-043

Investigating the *Plasmodium falciparum* Hsp90 chaperone complex, a potential antimalarial drug target

Chun Song Chua, Cher Siong Gan, Tiow Suan Sim

Department of Microbiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

Heat shock protein 90 (Hsp90) is a molecular chaperone that is essential for cellular processes. Hsp90 functions as a complex, together with other chaperones (e.g. Hsp70, Hsp40) and cochaperones (e.g. p23, Hop) in protein folding and activation. The list of Hsp90 client proteins is extensive and they include transcription factor p53 and important signaling kinases such as Raf-1, Src and MEK. As many of these proteins are involved in oncogenesis, inhibition of Hsp90 resulted in the disruption of their protein folding/activation states, leading to growth and developmental arrest in cancer cells. The selectivity of Hsp90 inhibitors towards cancer cells, as opposed to normal cells has been attributed to the conformation of Hsp90. Most Hsp90 in the normal cells are found to be in the non-complexed state. However, in cancer cells, Hsp90 was found to be present predominantly in the functionally active complexed conformation which has higher affinity for the inhibitors. This finding highlights the importance of acquiring knowledge on Hsp90 native conformation for selective inhibition and treatment.

In *Plasmodium falciparum*, the cytosolic Hsp90 homologue (PfHsp90) has been reported. Treatment of *P. falciparum* culture with an Hsp90 inhibitor, geldanamycin, arrested the parasite intraerythrocytic progression at various stages. This finding suggests the importance of PfHsp90 for development and the presence of a similar chaperone system in *P. falciparum*. Although targeting PfHsp90 is attractive in combating malaria, the direct extrapolation of using Hsp90 inhibitors as antimalarials may be premature due to the lack of understanding on the functional Hsp90 chaperone complex in *P. falciparum*. In the attempt to characterize PfHsp90, we present here the (a) identification of putative PfHsp90 protein folding complex via computational analyses, (b) cloning and expression of these recombinant proteins and (c) in vitro reconstitution of the complex and analyses of its ATPase activity.

P-044

Dynamic structural analysis of the electron transfer complex between Cytochrome *c* and Cytochrome *c* Oxidase revealed by ^{15}N relaxation measurements

Koichi Sakamoto[1], Masakatsu Kamiya[2], Kyoko Shinzawa-Itoh[3], Tomoyasu Aizawa[2], Makoto Demura[2], Keiichi Kawano[1], Shinya Yoshikawa[3], Koichiro Ishimori[1]

[1]Department of Chemistry, Graduate School of Science, Hokkaido University, [2]Division of Molecular Life Science, Graduate School of Life Science, Hokkaido University, [3]Department of Life Science, Graduate School of Life Science, University of Hyogo, Japan

Cytochrome *c* (Cyt *c*) mediates an electron transfer between Cytochrome bc_1 complex (Cyt bc_1) and Cytochrome *c* Oxidase (C*c*O) in the respiratory chain. The Cyt *c*-mediated electrons from Cyt bc_1 are used for the four-electron reduction of molecular oxygen to water molecules in C*c*O. Although the electron transfer reaction from Cyt *c* to C*c*O is one of the crucial processes in the oxygen reduction, its molecular mechanism is still unclear due to the lack of the structural information, particularly dynamic motion of the proteins, on the electron transfer complex. Here, we investigated the dynamic motion of Cyt *c* and the effects of the C*c*O binding by NMR relaxation measurements, and tried to characterize the specific electron transfer reactions between Cyt *c* and C*c*O. To examine the main chain dynamics of ferrous Cyt *c* upon binding to C*c*O, we performed Modelfree analysis for the backbone ^{15}N relaxation parameters (T_1, T_2, NOE) in the presence and absence of unlabeled C*c*O and estimated the order parameter S^2 which reflects the restriction of the internal motions and ranges from 0 (free motion) to 1 (completely restricted motion). Our analysis revealed that the averaged S^2 value of Cyt *c* (0.884) was higher than that of other proteins, corresponding to the restricted motions of the backbone in Cyt *c*. Interestingly, the internal motion was more restricted in the binding site to C*c*O (averaged S^2: 0.901), indicating that C*c*O recognizes the highly restricted regions of Cyt *c* to form the electron transfer complex. As we expected, addition of C*c*O further increased the S^2 values (0.991) for the C*c*O binding site on Cyt *c*, and the internal motion of the binding site was more restricted by binding of C*c*O. In addition, the S^2 values for the other parts of the protein were also unexpectedly enhanced, showing that the backbone fluctuation of Cyt *c* is entirely suppressed upon binding to C*c*O. The electron transfer from Cyt *c* to C*c*O is, therefore, mediated by formation of the "rigid complex" where the internal motion of the whole molecule of Cyt *c* is highly suppressed. Such restricted motion would characterize the specific electron transfer from Cyt *c* to C*c*O.

P-045

The elongated domains of L4 and L22 and their role in the ribosome function

Aikaterini Tsagkalia[1], Knud Nierhaus[2], Lasse Lindahl[3], Janice Zengel[3],
Theodora Choli-Papadopoulou[1]

[1]School of Chemistry, Laboratory of Biochemisrty, Aristotle University of Thessaloniki, Greece
[2]Max-Planck Institut for Molecular genetics, Berlin, [3]University of Maryland, Baltimore Country, USA

L4 and L22, proteins of the large ribosomal subunit, contain globular surface domains and elongated 'tentacles' that reach into the core of the large subunit to form part of the lining of the peptide exit tunnel. Mutations in the tentacles of L4 and L22 confer macrolide resistance in a variety of pathogenic and non-pathogenic bacteria. In E. coli only two erythromycin-resistant (ery-R) mutants have been characterized: strain N281 contains a deletion removing Met-Lys-Arg corresponding to codons 82・4 of L22, and strain N282 contains a change from lysine to glutamine at codon 63 of L4. To learn more about the roles of the loops in ribosome assembly and function, different constructs missing parts of this area, were designed. For L4 three mutants were used, the E61A L4 which carries a single amino acid substitution, the L4 loop1 which is missing 26aa (56-82) in this area and the L4 loop2 missing 36aa (46-82). As for L22 the mutants were the previously mentioned Ery L22, missing 3 amino acids (82-84), L22 loop1 with 14aa missing (82-95) and L22 loop2 which misses 17aa (82-98) from the area of the elongated 'tentacle'. The proteins were expressed as His-Tag, purified using Ni^{2+}NTA column and used in the total reconstitution experiments substituting the corresponding wild type ribosomal protein. The activity of the reconstituted 50S subunits was tested via poly(Phe) synthesis after the addition of the 30S subunit. The results indicated a higher sensitivity in the L4 mutants resulting in less active ribosomes. The ribosomes incorporating the L22 loop1 exhibited higher activity, pointing out that the area of the loop1 may not to be essential for ribosome assembly and activity. Overall the results highlight the important role of L4 and L22 in ribosome function and assembly, and indicate that a variety of changes in these proteins can mediate macrolide resistance.This research project is co-financed by E.U.-European Social Fund (75%) and the Greek Ministry of Development-GSRT (25%).

P-046

Phosphorylation of S5 ribosomal protein from mouse by Casein Kinase II is essential for its cellular traffic

Helen Papachristou[1], Christina Matragou[1], Zoe Karetsou[2], Thomais Papamarcaki[2], **Theodora Choli-Papadopoulou**[1]

[1]*School of Chemistry, Laboratory of Biochemisrty, Aristotle University of Thessaloniki,*
[2]*Laboratory of Biological Chemistry, Medical School, University of Ioannina, Greece*

S5 belongs to 40S subunit ribosomal proteins of the S7 family and is one of the early assembly. S5 according to its homology possesses an N-terminal extension possible target for phosphorylation. The phosphorylation sites was investigated after cloning within different vectors and by using confocal microscopy.The *in vitro* phosphorylation by Casein Kinase II verified the indication mentioned above regarding the N-terminal region as target. The first 37 amino acids as well as the remaining 38-204 were cloned in a pGFP vector in a fusion form with GFP protein. In addition mutations concerning the amino acids serine in position 24, S (24) and serine in position 34, S (34) were also performed and fused to GFP as well. In addition the phosphorylation of S5 was verified with IMAC followed by immunoblot.The data obtained thus far have shown that the entire protein is mainly localized within the nucleolus, but a smaller amount is also found in the cytoplasm. These results were also supported by biochemical experiments namely immunoblotting after separation of nucleolus from cytoplasm. Regarding the investigated truncated forms the 38-204 region is localized within the nucleus but not in the cytoplasm whether the 1-37 N-terminal region was not specifically localized. The N-terminal region is target for phosphorylation also by other Kinases for amino acids threonine 8 and threonine 14 and it has to be mentioned that by mutating both threonines 8 and 14 the protein was remained in the cytoplasm (unpublished results). Interestingly, the constructs with serine-24 and serine-34 mutations enlighted the importance of these two amino acids in regulating its cellular traffic. Namely it is found that serine-24 mutant is essential for entering the nucleoli and serine-34 keeping the traffic ‘under control’ in general. In conclusion, the N-terminal region of the protein seems to be highly important for cellular phosphorylation and cellular traffic of S5.

P-048

The antimicrobial peptide LL-37 inhibits biofilm formation of uropathogenic *E. coli*

Ylva Kai-Larsen[1], Milan Chromek[2], Asa Holm[3], Petra Luthje[3], Xiaoda Wang[4], Jan Johansson[5], Ute Romling[4], Annelie Brauner[3], Birgitta Agerberth[1]

[1]*Department of Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm, Sweden,*
[2]*Department of Paediatrics, Karolinska University Hospital Huddinge, Stockholm, Sweden,*
[3]*Division of Clinical Microbiology, Department of Microbiology, Tumor and Cell Biology, Karolinska University Hospital and Karolinska Institute, Stockholm, Sweden,*
[4]*Department of Microbiology, Tumor and Cell Biology, Karolinska Institute, Stockholm, Sweden,*
[5]*Department of Anatomy, Physiology and Biochemistry, Swedish University of Agricultural Sciences, the Biomedical Centre, Uppsala, Sweden*

Background: Antimicrobial peptides are multifunctional effector molecules of innate immunity and are included in the defence of the human urinary tract against bacterial infections. Uropathogenic *E. coli* often grow as multicellular communities, generating biofilm, which may offer advantages in comparison to free living cells (planctonic phase). For example, biofilm protects the bacteria from attacks of antimicrobial components. In *E. coli*, curli and cellulose are the main extracellular matrix components that promote biofilm formation.
Aims: To compare the resistance of *E. coli* to the human antimicrobial peptide LL-37 when grown as biofilm versus planctonic phase. Further, to analyse if LL-37 can inhibit biofilm formation of this bacterial strain and to elucidate the potential mechanism behind this inhibition.
Methods: Uropathogenic *E. coli* and isogenic mutants expressing or lacking curli and/or cellulose were tested for biofilm formation and viability. To study kinetics of curli fibril formation, the monomeric form of recombinant csgA, i.e. curli was utilized. The filaments were stained with the fluorescent probes congo red and thioflavin T, and were detected with confocal microscopy or a Tecan plate reader. Binding studies were performed with Biacore and secondary structure determination was conducted with circular dichroism spectroscopy.
Results: The MIC of LL-37 against uropathogenic *E. coli* in biofilm versus planctonic phase was 20 μM and 10 μM, respectively. We also showed that LL-37 can block the biofilm formation of this strain already at 2.5 μM. Binding analyses revealed a high affinity of LL-37 to csgA. In addition, we found a pronounced inhibition of csgA polymerization by LL-37 at a molar ratio of 1:1. The structure and the levels of csgA monomers remained stable in the presence of LL-37, supporting the inhibition of the polymerization.
Conclusions: LL-37 inhibits biofilm formation of *E. coli*, with the prevention of curli polymerization as one mechanism. This inhibition makes the *E. coli* more sensitive to antimicrobial components and is most likely involved in the defence mechanism against *E. coli* infections in the urinary tract.

P-051

A serine protease secreted by Staphylococcus aureus evokes a vascular permeability accompanied with production of pro-inflammatory cytokines

Jong Woo Park, Eu-Ri Jo, Jung Eun Park, Jung Sup Lee

Department of Biotechnology, BK21 Research Team for Protein Activity Control, and Research Center for Proteineous Materials, Chosun University, Gwangju 501-759, Korea

Staphylococcus aureus (*S. aureus*) is a ubiquitous Gram-positive bacterium responsible for a majority of skin infections as well as causing toxic shock syndrome. We have characterized a glutamate-specific serine endopeptidase (named VSPase) secreted by *S. aureus* sp. strain C-66 in terms of the proteolytic activation of prothrombin. In this study, the involvements of VSPase in the induction of inflammatory response and also in the enhancement of vascular permeability were examined. VSPase clearly increased the expression level of the genes for pro-inflammatory cytokines such as TNF-alpha and IL-1beta, and also up-regulated an inflammatory regulator cyclooxygenase-2 in the level of transcription, as determined by RT-PCR, ELISA, and Western blotting. VSPase could induce the degradation of IkappaB resulting in the translocation of NF-kappaB proteins into nucleus, as judged by Western blotting and super-shift assay with anti-p65 antibody. These results suggest that VSPase can activate NF-kappaB signaling pathway through the degradation of IkappaB proteins, leading the production of pro-inflammatory cytokines and an inflammatory regulator. Interestingly, wild-type VSPase could cause an increased vascular permeability on guinea pig system in a dose-dependent manner, whereas its mutant enzyme S237L thatis totally deficient in proteolytic activity could not, as examined by Miles assay. These results suggest that the vascular permeability caused by VSPase is absolutely dependent on its proteolytic activity. Taken together VSPase plays an important role in the expression of pro-inflammatory cytokines and inflammatory regulator through the activation of NF-kappaB and also can enhance a vascular permeability during the bacterial infection.

P-052

Isolation and characterization of a fibrinogenolytic enzyme from Macrovipera mauntanica snake venom

Eun Hee Lee, Jung Eun Park, Jung Sup Lee

Department of Biotechnology, BK21 Research Team for Protein Activity Control, and Research Center for Proteineous Materials, Chosun University, Gwangju 501-759, Korea

Snake venom contains various enzymes and peptides showing anticoagulant and fibrinogenolytic activities. In this study, a fibrinogenolytic enzyme was purified and characterized from crude snake venom of *Macrovipera mauntanica*. For the purification, Superdex 75 10/300 GL, Source Q, and MonoQ column chromatographic steps were employed in order. The enzyme was purified to homogeneity as judged by its migration profile in SDS-PAGE stained with Coomassie blue, and showed a molecular mass of about 27 kDa. The purified enzyme was composed of single polypeptide and its amino-terminal sequence was found to be N-QRFAPRYIEL-C, identical to that of fibrinolytic metalloproteinase from the venom of *Vipera lebetina*, except for first amino acid glutamine. Among substrate proteins tested, fibrinogen was the most susceptible one for the purified enzyme. The alpha and the beta chains of fibrinogen were completely digested by the enzyme until 20 min and 4 hr at 37^{o}C, respectively. Ca^{2+} ions could not enhance the enzyme activity. As shown in other fibrinogenolytic enzymes from snake venoms, the gamma chain of fibrinogen was resistant to the enzyme cleavage. The fibrinogenolytic activity of the purified enzyme could be inhibited by 1,10-phenanthroline and EDTA, but not by TLCK and aprotinin. The purified enzyme could prolong 2.2 times the fibrinogen clotting time (FCT), compared to that of non-treated control. These results suggest that the purified enzyme may be a Ca^{2+}-independent anti-coagulant metalloprotease possessing alpha- and beta-fibrinogenase.

P-053

Elucidating potential phospho-regulatory sites in Pfnek3, a *Plasmodium falciparum* protein kinase, to rationalize its cellular activation

Huiyu Low, Tiow Suan Sim

Department of Microbiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

A diverse array of cellular events, including cell proliferation, differentiation and apoptosis, are controlled through reversible phosphorylation. This process is generally mediated by a group of signal transduction enzymes known as protein kinases. As such, protein kinases have been implicated in the regulation of crucial cellular functions. To date, cellular regulation in *Plasmodium falciparum*, the protozoan parasite that causes the most lethal form of human malaria, remains unclear. Therefore, there has been an increasing interest in plasmodial kinases in an effort to explore their potential roles in mediating crucial cell regulatory events in the parasite. Pfnek3, a *P. falciparum* NIMA-like kinase, was previously shown to activate a mitogen-activated protein kinase homologue (Pfmap2) via phosphorylation at the latter's activation site. Concomitantly, in an independent study, Pfmap2 was demonstrated to be fundamental for the completion of intra-erythrocytic schizogony of the parasite. In view of the above observations, it was proposed that Pfnek3 is an upstream regulator of Pfmap2 and that signaling pathways involving Pfnek3-Pfmap2 interaction may play a role in controlling cellular functions in *P. falciparum*. Currently, the regulation of Pfnek3 activity is not well understood. Nevertheless, it has been reported earlier that active Pfnek3 undergoes autophosphorylation *in vitro*. Since activation of protein kinases is often correlated with phosphorylation of key amino acid residues located within their kinase domains, it is conceivable that Pfnek3 could be activated via similar mechanisms. Adopting a combination of bioinformatics tools, potential phosphorylation sites in Pfnek3 were predicted. In total, four serine residues, S221, S222, S225 and S226, situated along a loop on the surface of the kinase domain were found to be potential phosphorylation sites. Identifying plausible phospho-regulatory sites in Pfnek3 would enable subsequent verification by mutational analyses to assess their influence in modulating enzyme activity of Pfnek3.

P-055

Role of C-terminal region of chaperonin GroEL: Identification of the functionally critical amino acid segment

Yasushi Kawata[1,2], Kodai Machida[2], Akane Kono-Okada[1], Kunihiro Hongo[1,2], Tomohiro Mizobata[1, 2]

[1]Department of Chemistry and Biotechnology, Graduate School of Engineering, Tottori University, Japan, [2]Department of Biomedical Science, Graduate School of Medical Science, Tottori University, Japan

The chaperonin GroEL (14-mer) from *Escherichia coli* binds denatured proteins and facilitates their folding *in vivo* and *in vitro* by encapsulating them within an isolated cavity formed in cooperation with the co-chaperonin GroES (7-mer). The final 23 residues (^{526}KNDAADLGAAGGMGGMGGMGGMM^{548}) in the C-terminal region of the GroEL are invisible in crystallographic analyses due to high flexibility. In order to clarify the functional role of these residues in the chaperonin mechanism, we generated and characterized C-terminal truncated, double ring and single ring mutants of GroEL. The ability to assist the refolding of substrate proteins, rhodanese and malate dehydrogenase, decreased suddenly when 23 amino acids were truncated, indicating that a sudden change in the environment within the central cavity had occurred. From further experiments and analyses of the hydropathy of the C-terminal region, we focused on the hydrophilicity of the sequence region ^{526}KNDAAD^{531}, and generated two GroEL mutants where these residues were altered to a neutral hydropathy sequence (^{526}GGGAAG^{531}) and a hydrophobic sequence (^{526}IGIAAI^{531}), respectively. Very interestingly, the two mutants were found to be defective in function both in vitro and in vivo. Deterioration of function was not observed in mutants where this region was replaced by a scrambled (^{526}NKADDA^{531}) or homologous (^{526}RQEGGE^{531}) sequence. This finding demonstrated that the hydrophilicity of this sequence was important. These results highlight the importance of the hydrophilic nature of ^{526}KNDAAD^{531} segment in the flexible C-terminal region for proper protein folding within the central cavity of GroEL.

P-056

Study on structural and functional roles of the C-terminal α-helix of calmodulin

Chihiro Kitagawa[1], Satoshi Nagaya[1], Kumiko Nakamura[1], Ryosuke Doi[1], Akiko Nakatomi[2], Michio Yazawa[2]

[1]Department of Chemistry, Graduate School of Science, Hokkaido University, [2]Division of Cellular Life Scisence, Faculty of Advanced Life Science, Hokkaido University, Japan

Calmodulin is a small eukaryotic Ca^{2+}-binding protein, which modulates the function of target enzymes in response to the intracellular Ca^{2+} levels. This highly conservative protein consists of two similar domains, each containing two helix-loop-helix Ca^{2+}-binding motifs called EF-hands. The bundles of four helices constituting the two EF-hands in each domain are well-packed in the apo state, and Ca^{2+} binding takes place with a large conformational rearrangement of these helices. Consequently, hydrophobic residues are exposed to the molecular surface forming a large cluster on each domain. The resulting hydrophobic clusters serve as sites for target enzyme binding. Properties of calmodulin from yeast (*Saccharomyces cerevisiae*), however, are different from those of others. Yeast calmodulin binds only three moles of Ca^{2+} due to defects in the region corresponding to the C-terminal fourth EF-hand. The most C-terminal α-helix of yeast calmodulin is involved in target activation, since a mutant protein with a deletion of the C-terminal three residues is not active, while exhibiting complete three Ca^{2+} binding with higher affinity. Role of the C-terminal α-helix on the structure and function of calmodulin was evaluated in this work using vertebrate calmodulin. Prokaryote expression plasmid for chicken calmodulin was manipulated to yield mutant proteins with deletion of the C-terminal residue at each step. Mutant proteins were evaluated for the secondary structure (CD measurements), Ca^{2+} binding and target enzyme activation (activation of calcineurin). Deletion of three residues did not affect the structure and enzyme activation. Deletion of the four residues resulted in slight decrease in the enzyme activation without significant effect on CD spectrum. Mutant with eight residue deletion did not show any activation of calcineurin, and its secondary structure was affected. Other mutants with deletion of five to seven residues will be characterized and the results will be presented with their Ca^{2+} binding properties. Role of the C-terminal helix will be discussed considering the results for yeast and vertebrate calmodulins.

P-057

Down-regulation of boar PDI-P5 having thiol reductase and antichaperone activities during the last stage of epididymal sperm maturation

Kuniko Akama[1,2], Tomoe Horikoshi[1], Atsushi Sugiyama[1], Aoi Akitsu[3], Nobuyoshi Niwa[3], Atsushi Intoh[3], Michiko Sugaya[2], Kazuo Takei[3], Noriaki Imaizumi[3], Rena Matsumoto[2,4], Hitoshi Iwahashi[2, 5], Shin-ichi Kashiwabara[6], Tadashi Baba[6], Megumi Nakamura[7], Tosifusa Toda[7]

[1]Department of Chemistry, Graduate School of Science, Chiba University, [2]Graduate School of Science and Technology, Chiba University, [3]Department of Chemistry, Faculty of Science, Chiba University, [4]International Patent Organism Depositary, National Institute of Advanced Industrial Science and Technology, [5]Health Engineering Research Center, National Institute of Advanced Industrial Science and Technology, [6]Graduate School of Life and Environmental Sciences, University of Tsukuba, [7]Research Team for Molecular Biomarkers, Tokyo Metropolitan Institute of Gerontology, Japan

In mammalian spermiogenesis, sperm mature to get fertility during the epididymal transit. We investigated expression profiles of proteins from boar epididymal caput, corpus, and cauda sperm using two-dimensional gel electrophoresis, peptide mass fingerprinting, and Western blotting analysis. We found that among 15 proteins identified the homologous protein of human protein disulfide isomerase-P5 (PDI-P5/ERp5/PDIA6) decreased less than 40% during the transit from epididymal corpus to cauda. The results of cloning and sequencing of boar PDI-P5 precursor confirmed this observation, and indicated that the mature PDI-P5 without the N-terminal signal sequence was expressed in the epididymal sperm. Boar recombinant mature PDI-P5 expressed in *Escherichia coli* showed thiol-dependent disulfide reductase activity assayed with insulin precipitation method, and antichaperone activity to inhibit oxidative refolding of lysozyme *in vitro*.
It has been reported that PDI-P5 enables shedding of tumor-associated natural killer group 2, member D ligands, thereby promoting tumor immune evasion, and promotes tumor cells invasion and metastasis by activating avian erythroblastic leukemia viral oncogene homolog 2 (ErbB2, neuro/glioblastoma derived oncogene homolog) and phosphoinositide-3-kinase pathways, and subsequently by stimulating RhoA and beta-catenin.
Together with these data, our results provide insight into the molecular basis of boar sperm maturation during epididymal transit.

P-059

Transmembrane peptides are useful tools for study of membrane proteins function

Daisuke Sugiyama[1], Satoshi Osada[1], Ryo Hayashi[1], Hiroshi Yamaguchi[1], Masoud Jelokhani-Niaraki[2], Ichiro Fujita[3], Hiroaki Kodama[1]

[1]Department of Chemistry, Faculty of Science and Engineering, Saga University, [2]Department of Chemistry, Wilfrid Laurier University, [3]Department of Pediatrics, Faculty of Medicine, Saga University, Japan

Receptors, translocators, channel proteins and membrane enzymes exist as integral membrane proteins upon cellular surface. They are known to function as dimers or even higher oligomers. It is reported that transmembrane (TM) peptides derived from integral membrane protein modulated dimerization and consecutive function of parent proteins. Many groups have studied transmembrane domains of G protein-coupled receptors (GPCRs) and other integral membrane proteins. In this study, we examined characteristics of TM domains of GPCRs and channel proteins.

Formyl peptide receptor, a superfamily of seven TM proteins are expressed on human neutrophils as the chemotactic peptide binding receptors. To reveal the function of TM domains, TM peptides derived from human formyl peptide receptor were synthesized, and their biological activities were evaluated on human neutrophils. Synthetic peptides exhibited no agonistic or antagonistic activities on superoxide anion production for human neutrophils. However, human neutrophils treated with TM peptides produced 4-folds superoxide anion compared with intact cells.

Mitochondrial uncoupling proteins, the six individual helical TM domains are known to transport anions, such as Cl^-, in addition to H^+ transport. To elucidate the anion channel properties and conformation of individual TM peptides of human uncoupling proteins, six peptides corresponding to each TM domain were synthesized. Consequently, it was found that all six TM peptides formed helical structures in phospholipid membranes, and the second TM peptide exhibited stable channel activities. Second TM domain is a plausible candidate to line the ion-conducting path of the uncoupling proteins channel.

These studies suggested that TM domains of formyl peptide receptor and uncoupling proteins can regulate the function of priming effect and ion transportation, respectively. So, the current results shows transmembrane peptides are useful tools for study of membrane proteins function.

P-060

Stoichiometry and interaction of proteins in the wedge assembly pathway of bacteriophage T4

Moh Lan Yap, Shuji Kanamaru, Fumio Arisaka

Department of Biomolecular Process Engineering, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, Japan

The assembly pathway of the tail of bacteriophage T4 is strictly ordered through sequential interactions of proteins. A central hub and six wedges combine to form a hexagonal tail baseplate. Seven gene products gp11, gp10, gp7, gp8, gp6, gp53, and gp25 associate sequentially in this order to form a wedge. Gp11 is an exception of this ordered sequential assembly pathway, where it can be added at any stage of the assembly pathway, indicating that its binding site is on the periphery of the wedge. Gp11 interacts with gp10 and forms an initial complex of the wedge, followed by the interaction of gp7 giving a complex of 3:3:1 stoichiometry. Domain III and domain IV of gp10 are shown to tightly interact with gp11 and the C-terminal of gp7, respectively.

In the present study, we aim to assemble the wedge unit in an *in vitro* system in order to investigate the interaction of the proteins and their conformational changes upon the interaction. Gp8, whose 3D structure has been determined, is a dimeric protein which is suggested to associate with the above-mentioned complex along the assembly pathway. Based on the X-ray structure, the C-terminal of gp8 is predicted to interact with gp7 in the wedge. Hence, three expression systems, namely that of gp8 with His-tag at the N-terminus (gp8N-His_6), gp10 and gp7 were constructed. These three proteins were expressed independently and the cells were mixed together in the step of sonication. Gp7 and gp10 were eluted together with gp8N-His_6 forming a complex in the nickel-affinity chromatography. This complex was purified through a series of column chromatographies. Sedimentation velocity analysis showed that a complex with a sedimentation coefficient of 12.0S is formed. Conversion of c(s) to c(M) revealed that this complex has a molecular weight of 402,000. This result indicated that it is a hetero-hexameric complex with the stoichiometry of 3:1:2 or $(gp10)_3(gp7)_1(gp8)_2$. In order to identify the interacting regions of gp8 with the other proteins in the complex, limited proteolysis of each protein and the complex is under way.

P-061

The novel methodology to tune the protein-protein interaction by ligand.

Kumiko Suzuki, Toshihisa Mizuno, Toshiki Tanaka

Department of Material Science, Graduate School of Engineering, Nagoya Institute of Technology, Japan

The protein-protein interaction presides the various biological events in life. Toward the understanding of their functions and networks, various techniques to regulate the specific protein functions are developed and applied so far. Here we examined another methodology to tune the protein-protein interactions *via* coiled-coil module assembly. Ligand-triggered folding of coiled-coil modules accompanying the large structural transition is potential tuning module for various natural proteins. So far we introduced these modules for single domain proteins, such as RNase T1 and GFP, as a ligand-dependent domain, and succeeded to manipulate their protein functions. Here we utilized this methodology to manipulate the protein-protein interactions. Bacteriophage T7 lysozyme binds T7 RNA polymerase at its allosteric site and this interaction inhibits the RNA synthesis of T7 RNA polymerase. We designed T7 lysozyme mutant fusing the ligand-dependent coiled-coil module between Pro^{23} and Ser^{24}. Since the folding of coiled-coil module is altered from the random-coil to helical bundled structure by the additive ligand peptide, the refolding of T7 lysozyme moiety should be induced. Thus the abolished inhibition activity of T7 lysozyme was restored. In other words, the protein-protein interaction was turned on by the ligand. We believe that this methodology has generality for tuning various protein-protein interactions with external ligands.

P-062

Structure and function of calmodulin immobilized on solid substrate studied by in situ ATR-IR and SFG spectroscopies -Ca^{2+} induced binding of mastoparan and structure change of interfacial water-

Tatsuhiko Adachi[1], Hidenori Noguchi[1], Akiko Nakatomi[2], Michio Yazawa[2], Kohei Uosaki[1]

[1]Department of Chemistry, Faculty of Science, Hokkaido University, [2]Division of Cellular Life Science, Graduate School of Life Science, Hokkaido University, Japan

Vibrational spectroscopy is one of the most powerful methods for the functional and conformational studies of proteins and other biomaterials. Here, we describe results of two investigations. Calmodulin (CaM), which is one of the Ca^{2+} binding protein, immobilized on a chemically modified gold surface by binding its histidine-tag to surface attached nickel-chelating nitrilo-triacetic acid (Ni-NTA), maintaining its activities. Association and dissociation of target peptide mastoparan(MP) with the immobilized CaM was monitored in real time using in situ ATR-IR spectroscopy by varying the concentration of MP to estimate the binding constant of MP with immobilized CaM. The binding constant was 4 orders of magnitude smaller than homogeneous system, suggesting that the conformational change of CaM was sterically hindered by surface immobilization. Mg^{2+} was also demonstrated to play an important role for the dissociation of MP with CaM.The structure change of interfacial water at CaM surface due to Ca^{2+} induced conformational change was also investigated by using sum frequency generation (SFG) spectroscopy, which is particularly useful to study the structure of interfacial water where the presence of large amount of bulk water makes the measurement of interfacial water by other vibrational techniques very difficult.

P-063

Dynamic assembly properties of nonmuscle myosin II isoforms revealed by fluorescence cross-correlation spectroscopy

Mariko Mitsuhashi[1], Hiroshi Sakata[2], Masataka Kinjo[2], **Masayuki Takahashi**[1], Michio Yazawa[2]

[1]Department of Chemistry, Faculty of Science, Hokkaido University, [2]Division of Cellular Life Science, Faculty of Advanced Life Science, Hokkaido University, Japan

Nonmuscle myosin II is responsible for a number of cellular motile processes. To function in the cell, nonmuscle myosin II molecules assemble into filaments through their C-terminal tails. In mammalian cells, there are three isoforms of nonmuscle myosin II (IIA, IIB and IIC). To function effectively, it is reasonable to consider that they assemble in an isoform-specific mode to form homo-filaments in cells. However, little is known about the nature of myosin II isoform composition in filaments. Fluorescence correlation spectroscopy (FCS) is an extremely sensitive method based on the fluctuation analysis of fluorescence intensity coming from a small and fixed volume element to detect and characterize fluorescent molecules in a homogeneous solution. Dual color fluorescence cross-correlation spectroscopy (FCCS) can detect the coincidence of two molecules labeled with spectrally distinct fluorescent probes. In order to obtain new insight into the assembly properties of nonmuscle myosin II, we carried out the single molecule level analyses of FCS and FCCS experiments using C-terminal rod fragments of the IIA and IIB isoforms (ARF296 and BRF305) labeled with fluorescent probes, Alexa-633 and Alexa-488, respectively. Firstly, we confirmed that these techniques could distinguish between the assembled and disassembled states of myosin II rod fragments. Secondly, we clarified that the rod fragments assemble randomly without any distinction between the IIA and IIB isoforms, and the exchange of subunits (rod fragments) proceeded rapidly among the pre-formed homo-assemblies without distinction of isoforms. We then analyzed the effects of Mts1 (S100A4) on the assembly properties of myosin II isoforms. Mts1 is a member of S100 family protein and is known to bind myosin IIA specifically in the presence of Ca^{2+}. We found that addition of Mts1 specifically stripped ARF296 away from the pre-formed hetero-assemblies, and consequently, homo-assemblies of BRF305 were formed. Further addition of EGTA recovered the hetero-assemblies again. FCCS method allowed us to analyze directly the dynamic assembly-disassembly processes of two different isoforms in one chamber.

P-064

Surface plasmon resonance and mass spectrometric analysis of protein interaction in heme catabolism

Hiroshi Sakamoto[1], Yuichiro Higashimoto[2], Masakazu Sugishima[2], Masato Noguchi[2]

[1]*Department of Bioscience and Bioinformatics, Kyushu Institute of Technology,* [2]*Department of Medical Biochemistry, Kurume University School of Medicine, Japan*

Heme oxygenase-1 (HO-1) catalyzes the physiological degradation of heme to biliverdin, carbon monoxide and free iron by utilizing molecular oxygen and electrons donated from NADPH-cytochrome P450 reductase (CPR). Biliverdin is subsequently converted to bilirubin by biliverdin reductase (BVR). Using Surface Plasmon Resonance (SPR) technique, we found that HO-1 associated with CPR more tightly in the presence of NADP (K_d = 0.5 μM) than in its absence ((K_d = 2.4 μM). BVR competitively inhibited the binding of HO-1 to CPR. Site-directed mutagenesis study showed that Lys-149 and Arg-185 of HO-1 are important in both the HO activity and its association with CPR. Protein-protein interaction of three enzymes involved in heme degradation was also investigated by acetylation of HO-1 in the absence and presence of CPR or BVR followed by peptide mass fingerprinting. Nine acetylated peptides were identified by MALDI-TOF mass spectrometry in the tryptic fragments obtained from HO-1 acetylated without the reductases. The presence of CPR prevented HO-1 from acetylation of lysine residues, Lys-149 and Lys-153. On the other hand, the presence of BVR showed no protective effect on the acetylation of HO-1. The interaction of HO-1 with CPR or BVR is discussed based on SPR and mass spectrometric analysis and on molecular modeling.

P-065

Prion protein structural distinctiveness characterized by octapeptide tandem repeat

Yuji Horiuchi, Eriko Hattori, Satoru Yokotani, Ayami Matsushima, Takeru Nose, Yasuyuki Shimohigashi

Laboratory of Structure-Function Biochemistry, Department of Chemistry, The Research-Education Centre of Risk Science, Faculty and Graduate School of Sciences, Kyushu University, Fukuoka 812-8581, Japan

The prion protein (PrP) is responsible for bovine spongiform encephalopathy (BSE), one of so-called prion diseases. Its normal cellular form designated as $PrP^{C}$is believed to possess a molecular mechanism to transform the protein structure into a refolded form, the scrapie PrP^{Sc}. From the amino acid sequence of prion protein, it is unable to make a guess at the transformation from PrP^{C} to PrP^{Sc}. Since the structural change of PrP is quite unique, we first simply speculated that its primary structure must have a unique amino acid composition. Analysis using a newly developed program for radar-chart deviation analysis has identified an abnormality or irregularity of the N-terminal flexible domain. Aromatic amino acids Trp and His together with Gly are abnormally abounding in this N-terminal domain, in which octapeptide GQPHGGGW is connected four times in tandem. This tetrarepeat structure has been suggested to be essential for the prion protein not only to play an intrinsic functional role under the physiological condition, but also to bring on structural abnormalities in prion disease.

In the present study, we analyzed in detail the structural distinctiveness of this tetrarepeat structure, GQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGW, by several different spectroscopic methodologies such as the fluorescence spectrometry, MALDI-TOF mass spectrometry, and quartz-crystal microbalance (QCM) measurement. One of the most prominent structural characteristics is a sequential assembly of aromatic amino acids His and Trp alternately every four residues. His and Trp can make a strong $\pi - \pi$ stacking interaction intermolecularly or themselves. Using a series of oligomeric repeat peptides Ac-$(GQPHGGGW)_n$-NH_2 (n = 1 - 4) and N-terminal PrP proteins, we could identify and quantify oligomeric molecular interactions mediated through the His and Trp residues between peptides and/or proteins. All the results have suggested that the tetrarepeat peptide region is important for a specific molecular interaction between prion proteins, perhaps between PrP^{Sc} and PrP^{C}.

P-066

Comprehensive proteomic analyses of human heterochromatin protein 1 (HP1) interacting factors

Ryu-suke Nozawa[1], Koji Nagao[2], Osamu Iwasaki[1], Chikashi Obuse[1]

[1]*Faculty of Advanced Life Science Hokkaido University,* [2]*G0 Cell Unit, Initial Research Project, Okinawa Institute of Science and Technology, Japan*

Heterochromatin protein (HP1) is a conserved small protein highly enriched in heterochromatic regions in the nucleus, and thought to play a role in both heterochromatic formation and transcriptional repression through an interaction with lysine-9 tri-methylated histone H3. We have previously shown that the kinetochore Mis12 complex associates with HP1 in human cells. Furthermore, human HP1 associates with the origin recognition complex and telomeres, implying a role in maintenance and inheritance of chromosomes. Human cells contain three subtypes of the HP1 protein, α, β and γ, which are thought to have distinct functions.As the first step to elucidate the distinct molecular roles for HP1 subtypes, we performed semiquantitative analysis of HP1 binding proteins using proteomic analysis with mass spectrometry. Flag-tagged HP1α, HP1β and HP1γ expressing cell lines were established, and we have currently identified more than 100 proteins including CAF1, TIF1 and Mis12 as HP1-interacting candidates. Proteins differed in their association with the HP1 subtypes: approximately half of the proteins associated with all three subtypes of HP1, whereas the remaining proteins preferentially associated with only one or two HP1 subtypes. This difference is consistent with distinct roles for each of the three HP1 subtypes. We are also analyzing the cell-stage specific association of HP1 subtypes with HP1 binding proteins. Interestingly, preliminary data showed that interactions between HP1 and many of the binding proteins were weaker in M phase compared to that in S phase, reflecting the dynamic regulation of heterochromatin throughout the cell cycle.

P-067

Ideal BRC peptide sequence for the inhibition of filament formation of human Rad51 protein

Julian Nomme[1], Yuya Asanomi[2], Vinh Tran[1], Kazuyasu Sakaguchi[2], **Masayuki Takahashi**[1]

[1]UMR6204 CNRS & Universite de Nantes, [2]Department of Chemistry, Faculty of Science, Hokkaido University, Japan

The human Rad51 (HsRad51) is a key element of homologous recombination and catalyzes strand exchange between two DNA molecules of identical sequence. HsRad51 thus plays a crucial role in DNA repair and DNA segregation for cell division, and is related to the resistance to radio- and chemo- therapies of cancer cells and their proliferation. HsRad51 is, therefore, an interesting target for anti-cancer treatment. Pellegrini and colleagues proposed by their crystallographic analyses of HsRad51 in complex with one of BRC motifs (BRC4) of BRCA2 tumor suppressor that the peptide could interfere with the filament formation of HsRad51(Pellegrini et al. Nature 420:287-293, 2002).We have experimentally showed that the 28 amino acid peptide derived from BRC4 motif efficiently interacts with the subunit-subunit interface of HsRad51 and prevents its filament formation on the DNA, the first step of strand exchange reaction (Nomme et al., Genes to Cells 13:471-481, 2008). To find out better amino acid sequence for the inhibition, we analyzed the interaction of peptide with HsRad51 by computing the binding energy based on the crystallographic structure. We observed that two phenylalanine residues at position 1524 and 1546 are important. In fact, the elimination or replacement with alanine of one of the residues abolished the inhibitory effect. By contrast, the peptide of 23 amino acids keeping the two phenylalanine residues retained the effect. We then searched the best amino acid sequence for the inhibition. For this purpose we built a molecular model of 8 BRC motifs of BRCA2 in the complex with HsRad51 based on the crystallographic structure of HsRad51-BRC4 complex and computed the binding energy for each residue in each motif. We then compared the result of all motifs and chose the amino acid, which provides the best binding energy at a given position, as the best amino acid. The study permitted us to propose substitution of 3 amino acids in the BRC4 peptide. The experimental analysis supported the proposition. Furthermore, the effect of each modification was additive. We gained more than 10-fold increase in efficiency by the modification.

P-069

Structural basis for dynamical interdomain movement of the selenocysteine-specific elongation factor SelB

Toyoyuki Ose[3], Nicolas Soler[2], Linda Rasubala[1], Kimiko Kuroki[1], Daisuke Kohda[1], Dominique Fourmy[2], Satoko Yoshizawa[2], Katsumi Maenaka[1]

[1]Division of Structural Biology, Medical Institute of Bioregulation, Kyushu University, [2]Laboratoire de Chimie et Biologie Structurales, ICSN-CNRS, [3] Faculty of Life Science, Hokkaido University, Japan

Selenocysteine (Sec) is an amino acid genetically-encoded by an unusual incorporation system, and thus called as the "21st" amino acid. The stop codon UGA turns to be Sec codon when the selenocysteine insertion sequence (SECIS) exists downstream of UGA on mRNA. In bacteria, Sec incorporation requires a unique Sec-specific elongation factor, SelB, which can specifically recognize $tRNA^{Sec}$ by its N-terminal EF-Tu-like domain and SECIS mRNA hairpin structure by its C-terminal extra domain. SelB functions in multiple translational steps: binding to SECIS mRNA and $tRNA^{Sec}$, delivery of $tRNA^{Sec}$ onto the A site, GTP hydrolysis, release from tRNA and mRNA. To date, the structural studies for the free form of the whole C-terminal domain (SelB-C, 377-634) consisting of four winged-helix motifs (WH1-4) and the mRNA SECIS recognition of a minimum mRNA-binding fragment (512-634, WH3-WH4) were reported. However, the structural characteristics that would explain this dynamical mechanism of selenocysteine incorporation on the ribosome remain to be revealed. We report x-ray crystallographic study of SelB-C in complex with the SECIS mRNA hairpin. The contacts observed between WH4 domain and the mRNA hairpin are similar to the one previously seen in the WH3-WH4-RNA complex. In contrast and surprisingly, the WH2-WH3 orientation of SelB-C is largely changed compared to its structure in absence of RNA. New interactions are established between the phosphate backbone of the neighboring partner of SECIS RNA and the positively charged residues lined on the WH2-WH3 interdomain surface of SelB-C. Two of these residues interact with those of the neighboring WH domains in the free form, stabilizing the L-shape structure kinked by the WH2-WH3 linker. This novel SelB-RNA interaction is sequence independent and may reflect the SelB-tRNA interaction and the SelB-rRNA recognition upon the SelB-ribosome complex formation. Furthermore, the N-terminal WH1 domain is not visible in two different crystal packings, suggesting that the WH1-WH2 linker is also highly flexible.

P-071

Quantitative analysis of oligomeric stability and transcriptional activity of tumor suppressor protein p53

Rui Kamada, Tomoko Terai, Takao Nomura, Yoshiro Chuman, Toshiaki Imagawa, Kazuyasu Sakaguchi

Department of Chemistry, Faculty of Science, Hokkaido University Japan

The tumor suppressor protein p53 is a 393 amino acid transcription factor that controls apoptosis and cell cycle arrest in response to DNA damage. The tetramer formation of p53 is essential for its efficient site-specific DNA binding and contributes to the ability of p53 to activate transcription from natural promoters. The tetramerization domain of p53 is located at the C-terminus and consists of a β-strand, a turn, and an α-helix. Approximately 50% of human tumors carry inactivating mutations in the *p53* gene. To date, 41 mutations were reported in 23 residues among 31 residues of the tetramerization domain. To determine the correlation between oligomerization state and transcriptional activity of mutant p53 proteins, we performed quantitative analysis for effect of mutation on the structure and compared transcriptional activity of the corresponding mutant proteins in living cell. Pro mutations in α-helix or mutations of Leu330 residue that located at the center of the hydrophobic core led destruction of tetrameric structure of p53. Three mutant peptides (F341C, L344R, and A347T) existed as a dimer unlike the wild-type. Full-length p53 proteins with the mutations abolished the transcriptional activity. Other mutant peptides could form the wild-type tetrameric structure at 10 μM concentration at 4ºC. Tetrameric structures of many peptides with mutations associated with human tumors were destabilized. In particular, mutations of amino acid residues in the hydrophobic core and of amino acid residues formed salt-bridge induced dramatically destabilization to the structure. These mutations also significantly reduced the transcriptional activity of p53 protein in cell. On the other hand, mutations of the residues accessible to solvent were less effective in destabilization for the tetrameric structures. The data demonstrated that there is a clear correlation between oligomerization state and transcriptional activity in mutant p53. Furthermore, the results suggested that the threshold of destabilization of the p53 tetrameric structure by mutation for dysfunction of p53 protein in cell could be very small.

P-072

A functional region of ELC required for the formation of 10S conformation in smooth muscle myosin

Tsuyoshi Katoh, Takanobu Taniguchi

Department of Biochemistry, Faculty of Medicine, Asahikawa Medical College Japan

Conformation and filament assembly of vertebrate smooth muscle myosin are regulated by the phosphorylation of its regulatory light chain (RLC) under physiological conditions. In the unphosphorylated state smooth muscle myosin forms the folded conformation (10S conformation) and stays as monomer, whereas in the phosphorylated state it does the extended conformation (6S conformation) that enables myosin to assemble into filaments. It had been thought that the essential light chain (ELC) was not involved in these phosphorylation-dependent events. However, we observed that an introduction of scallop adductor muscle myosin ELC into vertebrate smooth muscle myosin destabilized its 10S conformation. We thus studied on a region of the ELC required for the formation of 10S conformation for smooth muscle myosin using chimeric ELCs in which various parts of smooth muscle ELC were substituted by the corresponding sequences of scallop ELC. The chimeric ELCs were prepared using bacterial expression system and introduced into porcine aorta smooth muscle myosin. The conformations of these reconstituted myosins were examined by their elution times on gel-filtration HPLC and by the direct observation in electron microscopy. The chimeric ELCs with the domain 2 (residues 41-81) and residues 72-81 of scallop ELC destabilized the 10S conformation of the smooth muscle myosin most effectively whereas the other chimeric ELCs did not. The effects of chimeric ELCs with residues 72-77 and 78-81 of scallop ELC were further examined but either of them destabilized the 10 S conformation only partially. The results indicate the importance of residues 72-81 of ELC in the formation of 10S conformation. Based on the orientation of the side chains of residues 72-81 of ELC in the crystal structure of smooth myosin molecule, residues 72-77 are buried in the light chain molecule itself and interact with its other parts and that residues 78-81 are exposed, suggesting a possible interaction with the other subunit in the myosin molecule. The results suggest that both of them are required for the formation of the 10S conformation in smooth muscle myosin.

P-074

Elucidation of an unusual and catalytically critical hydrogen bond network in the catalytic center of human glutaminyl cyclase

Kai-Fa Huang[1], Yu-Ruei Wang[1], En-Cheng Chang[1], Tsung-Lin Chou[2], Andrew H.-J. Wang[1]

[1]*Institute of Biological Chemistry, Academia Sinica,* [2]*Institute of Biochemical Science, National Taiwan University, Taiwan*

QCs (glutaminyl cyclases; glutaminyl-peptide cyclotransferases, EC 2.3.2.5) catalyse N-terminal pyroglutamate formation in numerous bioactive peptides and proteins. The enzymes were reported to be involved in several pathological conditions such as amyloidotic disease, osteoporosis, rheumatoid arthritis and melanoma. The crystal structure of human QC revealed an unusual H-bond (hydrogen-bond) network in the active site, formed by several highly conserved residues (Ser(160), Glu(201), Asp(248), Asp(305) and His(319)), within which Glu(201) and Asp(248) were found to bind to substrate. In the present study we combined steady-state enzyme kinetic and X-ray structural analyses of 11 single-mutation human QCs to investigate the roles of the H-bond network in catalysis. Our results showed that disrupting one or both of the central H-bonds, i.e., Glu(201)...Asp(305) and Asp(248)...Asp(305), reduced the steady-state catalysis dramatically. The roles of these two COOH...COOH bonds on catalysis could be partly replaced by COOH...water bonds, but not by COOH...CONH(2) bonds, reminiscent of the low-barrier Asp...Asp H-bond in the active site of pepsin-like aspartic peptidases. Mutations on Asp(305), a residue located at the centre of the H-bond network, raised the K(m) value of the enzyme by 4.4-19-fold, but decreased the k(cat) value by 79-2842-fold, indicating that Asp(305) primarily plays a catalytic role. In addition, results from mutational studies on Ser(160) and His(319) suggest that these two residues might help to stabilize the conformations of Asp(248) and Asp(305) respectively. These data allow us to propose an essential proton transfer between Glu(201), Asp(305) and Asp(248) during the catalysis by animal QCs.

P-075

Analysis of stability for p53 tetramerization domain peptides derived from various species

Takao Nomura, Rui Kamada, Yoshiro Chuman, Kazuyasu Sakaguchi

Department of Chemistry, Faculty of Science, Hokkaido University, Japan

The tumor suppressor protein p53 plays a central role in cell cycle, development and differentiation by regulating the expression of target genes. Mutations of the tumor suppressor protein p53 are found in about a half of all human tumors. The tetramer formation of the p53 protein is essential for regulation of its activity. The human p53TD is comprised of β-strand (326-333), tight turn (334), and α-helix (335-356). Fourty-one mutations were reported in 23 residues among 31 residues of the tetramerization domain (TD). We recently demonstrated that these p53 mutations reduced the stability of the tetramer and resulted in the reduction of the transcriptional function as tumor suppressor protein. In the higher organisms, especially vertebrate, the p53 tetramerization domain was highly conserved. For example, the similarity between the human p53TD and zebrafish p53TD was 82 % and identity was 62 %.

In this study, we synthesized a series of p53TD peptides with sequences derived from several species including mammalian, bird, amphibian, and fish. Structures and stabilities of these p53TD peptides were analyzed by CD spectrometry and gel filtration in order to understand the effect of each substituted amino acid on the tetramer stability. In the gel filtration analysis, p53 peptide homologs were eluted at the tetramer fraction. The p53TD peptides except the fish homolog showed similar CD spectra to that of human p53TD at 4°C. The CD spectra of fish p53TD peptides showed lower ellipticity, but were distinctive of those of destabilized mutant peptides. Furthermore, we analyzed the thermal stability of each p53 homologs by monitoring the 222 nm of CD spectra at various temperatures. The p53TD peptides from sheep, guinea pig, tree shrew, and chicken exhibited the higher thermal stability than the human peptide. On the other hand, the fish peptides formed destabilized tetramers, and the amphibian p53TD peptide exhibited the moderate thermal stability. These data suggested that the stability of p53 tetramer structure had been increased through the evolutions. Moreover, the results suggested importance of the hydrophobic networks in stabilization of the p53 tetramer structure.

P-076

Site-directly ^{19}F-labeled collagen model peptides

Nozomi Sato[1], Kazuki Kawahara[2], Daisuke Motooka[1], Masamitsu Doi[3], Yuji Nishiuchi[4], Nobuaki Nemoto[5], Takashi Nakazawa[6], Yoshinori Nishi[1], Takuya Yoshida[2], Tadayasu Ohkubo[2], Yuji Kobayashi[1]

[1]Osaka University of Pharmaceutical Sciences, [2]Graduated School of Pharmaceutical Sciences, Osaka University, [3]Wakayama National Collage of Technology, [4]Peptide Institute Inc, [5]JEOL LTD, [6] Department of Chemistry, Nara women's University, Japan

The collagen molecule consists of three polypeptide chains, each of which takes left handed helical conformation. This structure reflects its unique amino acid sequence with the repetition of characteristic triplet, X-Y-Gly, where the X and Y position are commonly occupied by Pro and Hyp. We have investigated a series of synthetic peptides and demonstrated that they model collagen by showing that $(Pro\text{-}Pro\text{-}Gly)_n$ and its analogues with defined molecular weights take triple helical structure at lower temperature and undergo the conformational transition to single random coil structure.

Applying various physico-chemical techniques on these polytripeptides, we have explored the stabilizing mechanism of the collagen folds and focused on the contribution of an incorporated proline analogue in which a hydrogen atom is substituted by electron-withdrawing groups, i.e. hydroxyl or fluoro prolines. The transition temperatures of these substituted peptides vary widely depending on positions in the triplets and stereo chemistries of the substituted residues. During the investigation on the thermal stabilities of fluoroproline substituted peptides by NMR measurement, we observed a quite interesting behavior of the NMR signal of ^{19}F atom. Compared to ^{1}H or ^{13}C, ^{19}F chemical shifts are extremely sensitive to small environmental changes around the nuclei and show a very wide dispersion of chemical shifts.

Here we investigated the thermal transition of $(Pro\text{-}fPro\text{-}Gly)_7$ solving the longstanding problem of the severe overlapping of ^{1}H, ^{13}C and ^{15}N signals with which we had confronted previously in the analysis of the conformational transition. Although the ^{1}H NMR spectra monitoring this transition were simple because of poor peak separations, the ^{19}F spectra were very complicated with multiple peaks which might be ascribed to various conformational species. In order to trace the individual behaviors of each tripeptide unit of Pro-fPro-Gly we synthesized the site-directly ^{19}F-labeled peptides, $(Pro\text{-}Hyp\text{-}Gly)_m$-(Pro-fPro-Gly)-$(Pro\text{-}Hyp\text{-}Gly)_{6\text{-}m}$ ($m = 0 \sim 6$). The synthesis of these peptides and the results of ^{19}F NMR analyses will be discussed.

P-077

Effects of thermodynamic stability on expression level of p53 tetramerization domain peptides in *E.coli*

Hiromitsu Miyazaki, Rui Kamada, Takao Nomura, Yoshiro Chuman, Toshiaki Imagawa, Kazuyasu Sakaguchi

Department of Chemistry, Faculty of Science, Hokkaido University, Japan

The tumor suppressor protein p53 is a 393 amino acid transcriptional factor and induces cell cycle arrest or apoptosis in response to genotoxic stress. The tetramer formation of p53 is essential for its activity and the tetramerization domain of p53 is located at the C-terminal region. The unfolding process of the p53 tetramerization domain peptide (p53TD) was described as a two-state transition, in which the folded tetramer directly converts to denatured monomers. Mutations in the *TP53* gene have been found in more than 50% of human tumors. To date, 41 mutations were reported in 23 residues among 31 residues of the tetramerization domain. Recently, we have attempted to express various mutant p53TD peptides in *E. coli* to examine the stability of their tetramer. However, many mutant peptides were rarely expressed in *E. coli*. In order to evaluate the relation between the expression level and stability of tetrameric structure of the p53TD peptides, we performed a quantitative analysis of expression levels for mutant p53TD peptides. The p53TD peptides with tumor associated mutations were expressed in *E. coli* BL21 at 37°C, and the quantity of the expressed peptides was determined by measuring the intensity of p53TD bands against an internal standard band on the SDS-PAGE. Expression level of each mutant p53TD was calculated as a ratio of the mutant to the WT peptide. Mutant peptides with high stability of tetrameric structure were expressed in the *E. coli* at high protein level. In contrast, unstable peptides showed significantly low expression levels. Moreover, Expression of peptides that could not form any tetramers was not observed in the *E. coli*. Interestingly, expression levels of some unstable mutants were significantly increased when they were incubated at 25°C. The results indicated that there was a strong correlation between the expression level and stability of tetrameric structure for the p53TD peptides. Our study suggested that peptides and proteins with low stability should be in random-coil state and probably susceptible to peptidases or proteases in *E. coli*.

P-079

Diversity of bisphenol A-specific nuclear receptor ERR-γ due to the alternative pre-mRNA splicing

Yukimasa Takeda[1], Xiaohui Liu[1], Miho Sumiyoshi[2], Ayami Matsushima[1], Miki Shimohigashi[2], **Yasuyuki Shimohigashi**[1]

[1]Department of Chemistry, The Research-Education Centre of Risk Science, Faculty of Sciences, Kyushu University, [2]Division of Biology, Faculty of Science, Fukuoka University, Japan

Estrogen-related receptor gamma (ERRγ), one of 48 human nuclear receptors, has a fully active conformation with no ligand. We have demonstrated that ERRγ binds strongly bisphenol A (BPA), one of the nastiest endocrine disruptors, and thus retaining ERRγ's high basal constitutive activity. We also evidenced recently that a number of human fetal and adult tissues such as brain, heart, stomach, prostate, pancreas, and placenta, express several different ERRγ mRNA splicing variants. In some of these tissues, the low-dose effects of BPA have been supposed, and thus the adverse effects of ERRγ are very much suspicious. Although we did analyze that human placenta expresses predominantly one of ERRγ mRNA variant, little is known about the intrinsic molecular mechanism of ERRγ functions of as a transcription factor. In the present study, we demonstrated further the molecular multiplicity of ERRγ mRNAs and proteins, which must be correlated to their functional diversity.

By means of detailed analysis of human pancreas total RNA by reverse-transcription PCR, we finally identified total 34 mRNA splicing variants. These mRNAs eventually resulted in a production of 10 protein isoforms. Detailed real-time PCR analysis revealed that these isoforms exist in various tissues in a highly tissue-specific manner. Also, those were found in brain in a region-specific manner. ERRγ is in an activation conformation with no ligand, showing a high basal constitutive activity in the luciferase reporter gene assay. On the other hand, we found two distinct ERRγ isoforms having a wrecked ligand-binding domain (LBD) due to the lack of a peptide segment either 211-264 or 265-354. It should be noted that these LBD-wrecked isomers are considerably abundant (3-6% and 12-20%, respectively) in almost all the tissues, suggesting their physiological significance. These LBD-wrecked ERRγ analogs did neither exhibit constitutive activity nor bind BPA. All these results suggested a presence of novel regulatory mechanism in which ERRγ couples with its structural isomers produced by the alternative splicing.

P-080

A unique bimodal expression profile of intron-retained PERIOD splicing variants in the fruit fly *Drosophila melanogaster*

Yukimasa Takeda[1], Miho Sumiyoshi[2], Ayami Matsushima[1], Miki Shimohigashi[2],
Yasuyuki Shimohigashi[1]

[1]Department of Chemistry, Faculty of Sciences, Kyushu University, [2]Division of Biology, Faculty of Science, Fukuoka University, Japan

Animals usually exhibit a morning-evening bimodal distribution of locomotor activity in the circadian day/night cycle due to the respective independent oscillators. Molecular basis of the circadian clock is an auto-regulatory feedback loop, in which the PAS domain-containing protein PERIOD inhibits periodically a transcription of its own *period* gene. However, the comprehensive mechanism of such a bimodal oscillator system has never been elucidated especially on the molecular basis. We have identified a series of alternative splicing variants of insect and mammal *period* mRNA genes. Here we report that such gene *period* variants and the resulting PERIOD protein isoforms play intrinsic roles in the bimodal oscillator system.

In the fruit fly *Drosophila melanogaster*, three novel alternative splicing sites were identified in the successive introns at the 3'-region. Their splicings were found to provide eventually four different PERIOD proteins with different C-terminal structure. Quantitative real-time PCR analyses for each intron-spliced *period* mRNA revealed that the production of all of these mRNA isomers peaks at ZT 13. On the other hand, intron-retained *period* mRNAs were found to emerge at both ZT 5 and ZT 13 under the LD condition. This bimodal expression of *period* mRNAs is quite unique, since, unlike the evening peak (ZT 13), the morning peak (ZT 5) disappeared rapidly under the constant dark condition. The Western blotting analysis by using a monoclonal antibody specific for intron-retained PERIOD protein indicated that production of the intron-retained isoform is light-sensitive, but not thermo-sensitive. In contrast, a thermo-sensitive regulation is caused by another alternative splicing in the 3'-UTR as reported previously. As a result, the intron-retained PERIOD isoforms are likely involved in the morning oscillator, while the intron-spliced PERIOD isoforms are in the evening oscillator.

P-081

Intrinsically disordered structure of the C-terminal region in the *Helicobacter pylori* oncoprotein CagA

Takeru Hayashi[1], Masafumi Horio[1], Hideaki Higashi[1], Hiroyuki Kumeta[2], Fuyuhiko Inagaki[2], Masanori Hatakeyama[1]

[1]*Institute for Genetic Medicine, Hokkaido University,* [2]*Graduate School of Pharmaceutical Science, Hokkaido University, Japan*

Infection with *cagA*-positive *Helicobacter pylori* is associated with the development of gastric diseases such as atrophic gastritis, gastric ulcer and gastric adenocarcinoma. CagA, a 120-145 kDa protein encoded by the *cagA* gene, is delivered into gastric epithelial cells via the bacterial type IV secretion system. In the host cells, CagA undergoes phosphorylation at multiple tyrosine residues in its C-terminal EPIYA-repeat region that comprises multple EPIYA segments. Phosphorylated CagA then specifically interacts with SHP-2 phosphatase to disturb intracellular signals. CagA also associate with PAR1 kinase via the EPIYA-repeat region independently of CagA tyrosine phosphorylation. Since the CagA protein shares no sequence homology with any of known proteins, it is impossible to speculate tertiary structure of CagA based on its primary amino-acid sequence. Accordingly, to elucidate molecular basis for the interaction of CagA with its cellular targets, we sought to investigate tertiary structure of the purified recombinant CagA proteins. First, we performed limited proteolysis of full-length CagA to evaluate its possible domain structures and found that CagA was cleaved into a 95 kDa N-terminal fragment and a 35 kDa C-terminal fragment by V8 protease. The C-terminal fragment, which contains the EPIYA segments and is biologically active when expressed in mammalian cells, was highly purified to analyse its high-order structure by employing CD and NMR spectroscopy. The results of the study revealed that the EPIYA-repeat region was intrinsically disordered, consistent with the results obtained by using prediction programs that calculate disorder tendency of polypeptide. Notably, the purified CagA C-terminal fragment retained the ability to interact with PAR1, one of cellular targets of CagA, upon *in vitro* binding assay. These results suggest that structurally-disordered nature of the EPIYA-repeat region plays an important role in the interaction of CagA with its cellular targets.

P-082

Heme transfer mechanism from heme chaperone protein, CcmE, to apocytochrome c

Takeshi Uchida, Koichiro Ishimori

Department of Chemistry, Faculty of Science, Hokkaido University, Japan

c-type cytochromes are electron transfer proteins that are essential for the life of virtually all organisms. They characteristically have covalently-bound heme *via* thioether bonds to two cysteines in the protein. In Gram-negative bacteria, biogenesis of *c*-type cytochrome is conducted by a multiprotein complex system known as a cytochrome *c* maturation (Ccm) system. This system consists of 8 gene products (*ccmA-ccmH*). CcmE, which binds heme and delivers it to apocytochrome *c*, is called as a heme chaperone. In the previous study, covalent attachment of heme to cytochrome *c* was achieved in the presence of only CcmE, heme, and dithiothreitol [1]. However, the precise mechanism remains to be clarified. In this study, we applied UV/vis absorption and resonance Raman spectroscopies to the reaction of CcmE with apocytochrome c to understand the mechanism of heme transfer and covalent bond formation.

The heme transfer to apocytochrome *c* and covalent bond formation was monitored by UV/vis absorption spectroscopy. UV/vis absorption spectra were recorded at every 1 h intervals after addition of CcmE to apocytochrome c_{552}. The Soret band of CcmE shifted from 404 to 420 nm immediately after the reaction, which means that heme in the CcmE was reduced. Then, the Soret band gradually diminished with time, but no new bands appeared. This suggests that heme was released from CcmE, although it has covalent bond with heme, but not incorporated into apocytochrome *c*. We also tried apocytochrome c from horse and yeast (Saccharomyces cerevisiae), but obtained the same results as apocytochrome c_{552}. The CcmE we used has a C-terminal His_6-tag and thrombin recognition site. Because the C-terminal region of CcmE is important to recognize apocytochrome *c* [3], the extra C-terminal might inhibit the interaction of CcmE with apocytochrome *c*.

[1] Daltrop et al., *Proc. Natl. Acad. Sci. U.S.A.* **99**, 9703-9708 (2002).
[2] Karan et al., *J. Biol. Inorg. Chem.*, **7**, 260-272 (2002).
[3] Harvat et al., *J. Biol. Chem.*, **280**, 36747-36753 (2005).

P-083

New potential therapeutic targets for ovarian clear cell carcinoma

Noriaki Arakawa[1], Yusuke Masuishi[1], Yuko Yamanaka[1], Hiroshi Kawasaki[1], Etsuko Miyagi[2], Fumiki Hirahara[2], Hisashi Hirano[1]

[1]*Supermolecular Biology, International Graduate School of Arts and Sciences, Yokohama City University,* [2]*Department of Obstetrics and Gynecology, Yokohama City University School of Medicine, Japan*

Epithelial ovarian carcinoma is a morphologically and biologically heterogeneous disease, and is classified into four major histological types, serous, mucinous, endometrioid and clear cell adenocarcinoma (CCA). Among these types, CCA has a highly malignant potential as follows: the recurrence rate is higher even in early stage, the 3 and 5-year survival rates for patients are significantly lower, and the response rate to anticancer drugs including of platinum and taxane agents is lower. Therefore, it is necessary to find new therapeutic target for CCA. To identify proteins expressed specifically in CCA, we initially carried out a shotgun analysis using the iTRAQ reagents and compared the proteomic patterns of three ovarian cancer cell lines, OVISE and OVTOKO derived from CCA, and MCAS from mucinous adenocarcinoma. Of the 1105 proteins detected in the analysis, over 20 proteins were expressed higher >2-fold in OVISE and OVTOKO than MCAS. To investigate whether the increases in these expressions were observed in other CCA cell lines, we performed Western blot and quantitative RT-PCR analyses using five non-CCA and six CCA cell lines. Only protein level of superoxide dismutase 1, and both mRNA and protein levels in annexin IV (ANX4), laminin gamma 1 (LAMC1), N-myc downstream regulated gene 1 protein (NDRG1) and double cortin domain containing 2 (DCDC2) were increased preferentially in several CCA cell lines. Moreover, gene silencing of ANX4, NDRG1 and DCDC2 by RNA interfering elicited marked suppression of cell proliferation in CCA. These results suggest that these proteins might serve as therapeutic targets for ovarian CCA.

P-084

Tumor intracellular signal-targeting by application of specific substrate peptide for protein kinase Cα

Daisuke Asai[1], Jeong -H. Kang[2], Riki Toita[3], Tetsuro Tomiyama[3], Takeshi Mori[2,4], Takuro Niidome[2,3,4,5], Hideki Nakashima[1], Yoshiki Katayama[2,3,4]

[1]Department of Microbiology, St. Marianna University School of Medicine, [2]Department of Applied Chemistry, Faculty of Engineering, Kyushu University, [3]Graduate School of Systems Life Sciences, Kyushu University, [4]Center for Future Chemistry, Kyushu University, [5]PRESTO, J Japan Science and Technology Agency, Japan

In cancer gene therapy, the therapeutic genes must express specifically in target cancer cells to avoid any side effects. However, there is a serious issue since the most methods have insufficient ability to specifically recognize the diseased cells and distinguish them from normal cells. Recently, we proposed a unique strategy, called D-RECS (drug or gene delivery system responding to cellular signals), using polymer-based vehicles with a specific substrate peptide responsive to enzyme activity. In this concept, abnormally activated enzymes are used as a trigger of transgene expression. In the present study, we paid attention to a continuous activation of protein kinase Cα (PKCα) in several tumor cells, and we tried to achieve a specific transgene expression in PKCα-activated tumor cells. A peptide library containing 1772 kinds of peptides was designed and screened by MALDI-TOF-MS and conventional radiolabel assay. We found a peptide, FKKQGSFAKKK (Alphatomega) with high specificity for PKCα relative to other isozymes. Alphatomega was phosphorylated highly by lysates of tumor cell lines, but only a low level of phosphorylation was observed in normal tissue extracts, suggesting that it would be an appropriate cancer cell-specific indicator. We next synthesized two polymers [PPC(S)] and [PPC(A)] which carry Alphatomega or its negative control peptide (Ser to Ala substituent) by radical copolymerization between acrylamide and peptide macromonomer. The specific phosphorylation of [PPC(S)]/DNA complexes were confirmed by radiolabel assay. After the microinjection of [PPC(S)]/GFP-encoding DNA complexes into living cells, significant expression of GFP was observed. When the [PPC(S)]/DNA complexes were injected into the normal skin, the percentage of luciferase positive mouse decreased with the increase of [PPC(S)]. On the other hand, when the complexes were injected into the xenografted tumor, the injected mice showed luciferase expression at [PPC(S)] dose-dependent manner. In the case of [PPC(A)]/DNA complex, the expression was completely suppressed as expected. These results demonstrated tumor-specific gene expression sensing the activation of PKCα in tumor cells.

P-085

The effect of CDX1 transcription factor on human gastric epithelial cells

Yumiko Fujii[1], Daisuke Sasaya[1], Naoko Kamimura[2], Hiroyuki Aburatani[2], Masanori Hatakeyama[1]

[1]*Inst. Genetic Medicine, Hokkaido Univ.,* [2]*RCAST, Univ. Tokyo, Japan*

CDX1, a caudal type homeobox transcription factor, plays a crucial role in proliferation and differentiation of intestinal cells. Whereas physiological expression of CDX1 is restricted to the intestine, it is abnormally expressed in gastric mucosa with adenocarcinoma and intestinal metaplasia, a precancerous stomach lesion associated with *Helicobacter pylori* infection. We previously reported that CagA, a virulence factor of *Helicobacter pylori*, aberrantly induces CDX1 in gastric epithelial cells. To investigate the pathophysiological role of CDX1 expressed in the stomach, we established gastric epithelial cells that inducibly express Flag-tagged CDX1 with use the tet-off system. Total RNAs were extracted from the CDX1-inducible cells cultured for 24 h in the presence or absence of doxycycline and were subjected to a DNA microarray analysis. The results of the analysis revealed that CDX1 aberrantly induces expression of intestinal-specific genes in gastric epithelial cells. To further examine the target genes that are directly transactivated by CDX1 on the whole genome of the gastric epithelial cell, we explored genome-wide screening of promoter regions to which CDX1 actually binds. CDX1-bound genomic fragments were purified by chromatin immunoprecipitation with an anti-Flag antibody from the CDX1-inducible cells and were subsequently analyzed through a ChIP-chip analysis. We identified 167 genes as candidates for transcriptional targets of CDX1 in gastric epithelial cells from the results of ChIP-chip analysis and microarray analysis of mRNA expression. These candidates include genes encoding chromatin remodeling factors and transcription factors. The finding indicates that aberrant activation of CDX1 causes intestinal metaplasia and gastric adenocarcinoma through reprogramming gene expression in gastric epithelial cells. Elucidation of target genes for CDX1 systemically throughout the genome may contribute to our understanding of the transcriptional network by which cell fate is reprogrammed in gastric mucosa upon infection with *Helicobacter pylori*.

P-086

Binding of mouse prion protein to heparin

Kenta Teruya[1], Masahiro Wakao[3], Tomoaki Nishimura[2], Tomohiro Kimura[1], Yuji Sakasegawa[1], Yasuo Suda[3], Katsumi Doh-ura[1]

[1]Department of Prion Research, Graduate School of Medicine, Tohoku University, [2]SUDx-Biotech Corp., [3]Department of Nanostructure and Advanced Materials, Graduate School of Science and Engineering, Kagoshima University, Japan

Interaction between prion protein and endogenous glycosaminoglycans on the cell surface is proposed to play a key role in the infection and transmission of the prion, since sulfated glycans such as heparin and pentsan polysulfate exert anti-prion activities. However, interaction between prion protein and sulfated glycans is not fully evaluated. In this report, interaction between prion protein and heparin as a representative sulfated glycan was investigated to clarify which structural unit in heparin is responsible for the binding of prion protein, and which region of prion protein is responsible for the binding.Heparin is heterogeneous in composition and chain length, but there are a few known methods to destruct the partial structure of heparin specifically. Comparison of the anti-prion activities of the modified heparin prepared by two different methods revealed that a certain disaccharide unit is responsible to the potency. Then, "Sugar Chip" was prepared by immobilizing the disaccharide unit on a sensor chip to investigate which region of prion protein is responsible for the binding by using surface plasmon resonance (SPR) technique quantitatively. Because recombinant prion protein, murine rPrP23-231, exhibited a significant binding profile on the Sugar Chip, truncated domains of the recombinant prion protein were tested for their binding to the Sugar Chip. As a result, it was found that N-terminal domain of prion protein, 23-89, was responsible to the binding to the disaccharide unit in heparin. Finally, SPR analysis revealed that the interaction between the N-terminal domain and the disaccharide unit was competitively inhibited by pentosan polysulfate. These results suggest that this SPR-based assay system composed of the prion protein and the Sugar Chip might be utilized as either a facile anti-prion chemical screening method or a research tool to investigate the mechanism of anti-prion chemicals.

P-087

Proteomic analysis of serum from diabetic LEA/Sendai rats

Eri Takahashi[1], Tadashi Okamura[3], Kohei Ikari[2], Hisashi Hirano[1], Kazuki Yasuda[2], Yasushi Kaburagi[2]

[1]Supramolecular Biology, International Graduate School of Arts and Sciences, Yokohama City University, [2]Department of Metabolic Disorder, Research Institute, International Medical Center of Japan, [3]Department of Infectious Diseases, Research Institute, International. Medical Center of Japan, Japan

LEA/Sendai rats, a model of non-obese diabetes, have impaired insulin secretion as the main characteristic of diabetes. In this model rats, fibrosis of islets occurs with advancing age and at 24 weeks old, they develop mild diabetes with blood glucose level about 200 mg/dl two hours after oral glucose tolerance. To detect potential protein biomarkers for diabetes, we investigated serum from LEA/Sendai rats by 2D-DIGE and LC-MS/MS. BN rats were also examined as a control to diabetic rats.For 2-D DIGE analysis of serum samples, immunoaffinity column ProteomeLab^{TM} IgY-R7 removed seven abundant serum proteins; albumin, IgG, transferrin, haptogrobin, IgM, fibrinogen and alpha1-antitrypsin from serum of LEA/Sendai and BN rats. Proteins with altered expression between diabetic rats and non-diabetic rats at 8 weeks old and 16 weeks old were detected by 2D-DIGE and identified by Mass spectrometry. 24 proteins with altered expression between diabetic rats and non-diabetic rats were identified. In addition, 8 proteins with altered expression between 8 weeks old and 16 weeks old of LEA/Sendai rats were identified.Analysis of these proteins with differential expression may lead to the discovery of clinical biomarkers of early-stage diabetes.

P-088

Epitope analysis of kiwi fruit allergen actinidin

Tomohiro Nanri[1], Satomi Sugiyama[1], Yuri Kosuge[2], Yasuharu Itagaki[3], Hisashi Hirano[1]

[1]Yokohama City University International Graduate School of Arts and Sciences, [2]Nihon university Colleage of Biosourse Sciences, [3]Kanagawa Academy of Science and Technology, Japan

Since kiwi (*Actinidia deliciosa*) allergy was first described in 1981, there have been many reports on a wide range of symptoms from localized oral allergy syndrome to life threatening anaphylaxis. Proteins such as actinidin, thaumatin-like protein, kiwellin and 38-kDa protein are known as kiwi allergens. Among them, actinidin is the most abundant protein in kiwi fruit, and has a cysteine protease activity. In the present study, we analyzed the allergenicity of actinidin against twenty patients of kiwi allergy. Among them, six patients showed strong allergenicity against actinidin. To determine the protein domain containing epitope of actinidin, we purified actinidin from fruits of kiwi cv. Hayward by anion exchange column chromatography. Allergen activity was measured by ELISA. The purified actinidin was digested with *Staphylococcus aureus* V8 protease or endoprotease Lys-C. The resultant peptides were separated by reversed phase-high performance liquid chromatography. The immunoglobulin E (IgE)-binding activity of the obtained fractions was analyzed by competitive ELISA. Peptides in the fractions with the IgE binding activity were sequenced de novo by nano liquid chromatography-electrospray ionization quadrupole/time-of-flight mass spectrometry to identify the domain containing the epitope of actinidin. The domain containing the epitope consists of twenty amino acid residues, and was deduced to localize on the surface of the molecule, which is the hydrophilic regions of actinidin tertiary structure, suggesting that the epitope is localized in the site suitable for interaction with the IgE.

P-090

Identification and characterization of cholangiocarcinoma-associated antigens recognized by specific monoclonal antibodies.

Atit Silsirivanit[1], Sopit Wongkham[1], Chaisiri Wongkham[1], Chawalit Pairojkul[2], Vajarabhongsa Bhudhisawasdi[3], Kazuhiko Kuwahara[4], Nobuo Sakaguchi[4], Anthony Wilson[5], Megumi Nagayama[5], Daiki Kobayashi[5], Masayo Morifuji Wilson[5], Norie Araki[5]

[1]*Department of Biochemistry, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand,*
[2]*Department of Pathology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand,*
[3]*Department of Surgery, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand,*
[4]*Department of Immunology, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan,*
[5]*Department of Tumor Genetics and Biology, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan*

Cholangiocarcinoma (CCA) is a bile duct cancer with a high mortality rate, which has no specific markers for early diagnosis. The aim of our study is to develop monoclonal antibodies to detect CCA-associated antigens in patients・serum. Crude extracts from CCA tissues were used as the antigen source to immunize the Ganp-Tg Balb/c mice which produce high affinity antibodies (Sakaguchi et al., J. Immunol. 2005). Forty-one hybridoma clones which secrete specific monoclonal antibodies (mAb) against CCA antigens in patients・serum were firstly screened, and the two highest-potential clones, designated KKU-S27 and KKU-S121, were focused on. By Soy Bean Agglutinin (SBA)-captured ELISA, both KKU-S27 and KKU-S121 showed higher reactivity with the antigen in the CCA patient serum, suggesting that antigens for KKU-S27 and KKU-S121 are glycoproteins which have carbohydrate side chains containing GalNAc or Gal. Immunohistochemistry of CCA tissues using KKU-S27 and KKU-S121 revealed high reactivity to tumor bile ducts but lower activity with normal bile ducts. Immunofluorescent analysis with these mAbs also showed positive staining of CCA cell lines. The immunoblotting of sera and ascitic fluids from CCA patients showed that both mAbs react with the protein bands which were mobilized to higher molecular weights (more than 500 kDa) in a smearing pattern. In addition, the reactive bands in the CCA patient's serum lost their reactivity by alkaline treatment but not by N-glycanase, suggesting that both KKU-S27 and KKU-S121 react to the epitopes on the carbohydrate side chains that are linked to the core peptide by O-glycosylation. Proteomic analysis of the antigen protein in each immuno-precipitate revealed that both amino acid sequences identified are mucin family proteins. The proliferation rates of CCA cell lines were significantly decreased by treatment with both mAbs, suggesting that the KKU-S27 and KKU-S121 may be useful not only in CCA diagnosis, but also for therapeutic strategies although further investigation is necessary. The precise characters of the antigens for both mAbs will be reported. This project is co-supported by Khon Kaen University and Kumamoto University.

P-091

Study of molecular mechanisms in the development of highly metastatic human tongue cancer cells using combined differential transcriptomic and proteomic analysis

Masayo Wilson-Morifuji, Anthony Wilson, Daiki Kobayashi, Takashi Morikawa, Masayuki Tsubota, Norie Araki

Dept. Tumor Genet. Biol., Grad. Sch. Med. Sci., Kumamoto Univ. , Japan

To study the pattern of heterogeneous cancer development in human tongue cancer, we used a mouse model in which cancer cell lines of different metastatic abilities were mixed and transplanted, followed by observation of their growth and metastatic behaviors. First, we prepared two fluorescent-labeled human tongue cancer cell lines: a nonmetastatic cell line which stably overexpressed dsRed fluorescent protein (RFP-SQUU-A), and a highly metastatic cell line which stably overexpressed green fluorescent protein (GFP-SQUU-B). After mixing the RFP-SQUU-A and GFP-SQUU-B cells, we transplanted the mixture into the mouse tongues and observed the changes in growth and localization of each cell type for 42 days. The RFP-cells gradually disappeared from the tumor center and ultimately localized to the marginal regions exclusively, while the GFP-cells showed aggressive growth in the tumor center as well as lymph node metastasis. To determine the cellular factors related to these phenomena, the cell lines were subjected to differential proteomic (iTRAQ) and transcriptomic (DNA chip) analyses, followed by a study of the biological association to their networks involved in metastasis through an *in silico* analysis of the combined mRNA and protein data comparing metastatic SQUU-B with nonmetastatic SQUU-A. With the statistically highest score, we detected HIF (hypoxia inducible factor) signal pathway and Zo signal transduction as molecule-based pathways in signal networks that were specifically upregulated in metastatic SQUU-B cells. Detailed analysis of the HIF signal network by KeyMolnet showed that 30 (75%) of 41 proteins, which displayed higher expression in metastatic SQUU-B vs. nonmetastatic SQUU-A, were directly or indirectly related to HIF signal transduction. In addition, analysis by immunoblotting and immunocytochemistry revealed upregulation of the expression and activation of HIF-1α in SQUU-B. These results indicate that HIF signal transduction is the most important signaling mechanism in the metastatic SQUU-B cells, and that HIF and HIF-related cellular signals may be promising drug targets for the suppression of metastatic cancer development.

P-092

Discovery and validation of the protein biomarkers for tumor diagnosis: Differential analyses of the liquid chromatography/tandem mass spectrometry profiles from the tissue proteomes

Takao Kawakami[1], Atsushi Ogiwara[2], Kazuya Wada[2], Takashi Hirano[1], Harubumi Kato[3], Norihiko Ikeda[1]

[1]Department of Surgery, Tokyo Medical University, [2]Biomarker Mining Team, Medical ProteoScope Company, [3]International University of Health and Welfare Graduate School, Japan

In the biomarker development process with proteomics, the technology is generally applied to the early discovery stages in order to indicate several or more biomarker candidates. The following validation phases are essential to confirm quantitatively these candicates. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) has been extensively accepted for a proteome-wide profiling from a complex peptide mixture. Recent advances in this methodology have enabled differential display of the multiple two-dimensional peptide profiles of the LC separation and mass measurement. We developed an algorithm to compare and statistically analyze the peptide ion intensities among the two-dimensional profiles with alignment of the nonlinear fluctuation in the axis of LC elution time. Using the analytical platform, the quantitative proteome analyses of stage I primary lung adenocarcinoma tissues (N = 24) indicated the identification of the peptides from vimentin and myosin IIA with relationship between their up-regulation and the prognosis after the tissue resection. These results were confirmed at protein level with the immunological staining of the tissue specimen (N = 90) using the specific antibodies, which is one of the successful choices for the clinical utility of protein biomarkers. We will also discuss a data mining of the peptide profiles to interface the peptide-based discovery with the direct protein validation.

P-093

Development of the basic strategy for the comparative analysis of serum proteomes and its application in the discovery of hepatic injury biomarkers

Yoshio Kodera[3], Yusuke Kawashima[1], Tomoyuki Fukuno[1], Masahiro Maruhashi[1], Hiroki Takahashi[1], Takashi Matsui[1], Hiroyoshi Komatsu[4], Masanori Seimiya[5], Takeshi Tomonaga[5], Fumio Nomura[5], Tadakazu Maeda[1]

[1]Department of Physics, School of Science, Kitasato University, [2]Center for Disease Proteomics, School of Science, Kitasato University, [3]Clinical Proteomics Research Center, Chiba University Hospital, [4]Department of Clinical Laboratory Medicine, Faculty of Health Science Technology, Bunkyo Gakuin University, [5]Department of Molecular Diagnosis (F8), Graduate School of Medicine, Chiba University, Japan

As human serum provides a link between many human organs, tissues and cells, it is one of the most informative body fluids. The serum proteome includes 22 abundant proteins, which represent 99% of the mass of the proteinaceous content of human serum. Discovery of disease-specific proteins require quantitative analysis of the remaining 1% of the proteins. Therefore, in this study, we established a simple method of quantitative analysis for the serum proteome that had a high reproducibility.

Highly abundant albumin and IgG, which constitute about 80% of the total serum proteins, were depleted using a depletion column. Subsequently, the depleted serum samples were separated into 25 fractions using RP-HPLC. The fractionated serum proteins were then analyzed using two-dimensional electrophoresis. This method enables us to quantitatively detect disease-specific proteins with high accuracy. The method was also found to have a high reproducibility.

Direct analysis of serum proteins from patients is the most desirable method for searching for disease-specific proteins. However, since human serum proteins are quite variable and have individual differences, it is difficult to make data interpretations. We applied the established method to search for biomarkers in carbon tetrachloride induced hepatic injury rat serum. In a comparison of sera from two hepatic injury rats and two control rats, we found 106 protein spots that were different between the two groups, with 90 spots (55 proteins) successfully identified by LC-MS/MS. There are 13 proteins, such as sorbitol dehydrogenase, ornithine carbamoyltransferase, glutamate dehydrogenase, and glutathione S-transferase, that have previously been reported to be associated with liver disease-related proteins. The current results suggest that the established method was useful for searching for biomarkers in the serum, and furthermore, this simple method corresponds to the first step that is needed to discover the lower abundant disease–related proteins.

P-094

Novel chemical inhibitors for p53-inducible protein phosphatase PPM1D

Hiroaki Yagi, Yoshiro Chuman, Fumihiko Yoshimura, Keiji Tanino, Kazuyasu Sakaguchi

Department of Chemistry, Faculty of Science, Hokkaido University, Japan

p53-inducible protein phosphatase, PPM1D (Wip1, PP2Cδ), is a member of PPM1 family and controls cell cycle checkpoints in response to DNA damage. PPM1D is overexpressed in several human cancers and inactivates tumor suppressor protein such as p53 and p38. In addition, deficient of *PPM1D* gene causes a tumor resistant phenotype in mice, suggesting that *PPM1D* acts as oncogene. Furthermore, it was reported that inhibition of PPM1D expression can indeed reduce the viability of human tumor cells which gene of PPM1D is amplified. These findings indicate that PPM1D is a viable anti-cancer target, and provide justification for a search for PPM1D chemical inhibitors as potential anticancer drugs.

In this study, we carried out screening of PPM1D inhibitors from a unique chemical library, and identified a series of novel PPM1D inhibitors. One of the PPM1D inhibitors showed very strong inhibitory activity for PPM1D (IC_{50} = 400 nM), while the PPM1D inhibitor did not show significant inhibitory effect on PPM1A which is a member of PPM1 family. The result indicated that the compound has high specificity for PPM1D. We also analyzed the effects of the inhibitor to breast cancer MCF7 cells which gene amplification of PPM1D has occurred. The compound significantly induced phosphorylation at serine 15 of p53 protein in MCF7 cells. The result indicated that the compound inhibited the PPM1D phosphatase activity *in vivo*.

P-095

Characterization of cisplatin-resistant gastric cancer cell lines by quantitative protein kinetic analysis using high-density 'reverse-phase' protein lysate microarrays

Hironobu Noda, Satoshi Nishizuka, Kazushige Ishida, Go Wakabayashi

Molecular Therapeutics Laboratory, Department of Surgery, Iwate Medical University School of Medicine, Morioka, Japan

Clarifying the mechanisms of how cancer cells acquire drug resistance has been a critical issue in cancer chemotherapy. Here we present the isolation of a cisplatin (CDDP)-resistant population from the MKN45 gastric cancer cell line with a detailed functional evaluation at the protein level. Cell culture under continuous exposure of 16uM CDDP yielded 7 clones that seem to be resistant to the drug. One of the clones showed a 5-fold higher 50% growth suppression concentration with no cross-resistant among the 4 drugs tested including 5-FU, CPT-11, pacllitaxel, and oxialliplatin. To assess the functional difference between the parental line and the isolated resistant population, we employ a 'reverse-phase' protein lysate microarray system developed by the authors and Aushon BioSystems for quantitative protein monitoring in a high-throughput fashion. Using this system, protein kinetics as a function of drug concentration and time after administration can be elucidated. Preliminary Western blotting with several key proteins, including p53, was performed to determine the baseline characteristics of MKN45 in response to CDDP exposure. Concentrations of CDDP for the preliminary test were determined from a growth suppression assay, including levels of 0, 50, and 100% growth suppression. The p53 protein level increased in a drug dose-dependent manner; whereas, the p53-Ser15 level decreased over 24 hours of continuous drug exposure. These results suggest that active cell signaling associated with p53 takes place in response to CDDP exposure in a given concentration and time frame. To understand the cell signaling events, a number of protein kinetics need to be measured simultaneously in a quantitative and high-throughput manner. Here, we present protein kinetics associated with p53 and other proteins using the RPA system, and discuss how the system is useful for approaching questions in the context of drug resistance acquisition.

P-096

Analysis of molecular network kinetics induced by anticancer drugs for the identification of crucial molecular targets

Kazushige Ishida, Satoshi Nishizuka, Hironobu Noda, Go Wakabayashi

Molecular Therapeutics Laboratory, Department of Surgery, Iwate Medical University School of Medicine, Morioka, Japan

Anti-cancer agents are generally believed to affect particular parts of molecular networks, resulting in cell cycle arrest or apoptosis, but the molecular targets of most anti-cancer drugs in current use remain unclear. At the protein level, responses may vary depending on drug type, concentration, and mode of administration. To identify which molecular targets are crucial for responses to clinically used drugs, we developed a reverse-phase protein lysate microarray system (RPA) for measuring quantitative protein dynamics induced by drug exposure. For the preliminary experiment, three clinically used drugs, CDDP, 5-FU, and CPT-11, were administrated at four different concentrations and with two types of administration (sustained and tentative) to observe protein kinetics in a dose and time-dependent manner. Drug concentrations were at levels that would provide 0, 50, and 100% growth suppression, as determined by prior growth suppression assays. Among the proteins tested to date, p53, p21, CyclinD3 showed a dose-dependent protein expression following 24h-sustained administration for each of these 3 drugs, whereas, in a time course experiment, p53 protein showed a higher expression in response to CDDP and 5-FU exposure in a time-dependent manner, and a fluctuating pattern in response to CPT-11. Tentative administration (3h) resulted in a similar pattern of fluctuation in p53 protein expression in response to 5-FU and CPT-11, whereas the p53 expression increased in extended period of time in response to CDDP. These results may suggest that protein signaling differs depending on the type of drug and its mode of administration, even if the same phenotypic consequences, such as growth suppression, are induced. We are currently performing a high-resolution dose escalation, as well as time-course studies, to monitor protein kinetics in detail, using RPAs. We believe that a combination of RPA and bioinformatics approaches will allow drug molecular targets to be reliably determined.

Luncheon Seminar

Aug. 27, 12:30-13:30
Luncheon Seminar 1 -- Conference Hall A
(Sponsored by Applied Biosystems)

Luncheon Seminar 2 -- Conference Hall B
(Sponsored by Wako Pure Chemical Industries, Ltd.)

Aug. 28, 12:30-13:30
Luncheon Seminar 3 -- Conference Hall A
(Sponsored by Thermo Fisher Scientific Inc.)

Luncheon Seminar 4 -- Conference Hall B
(Sponsored by Nihon Waters K.K.)

Aug. 29, 12:30-13:30
Luncheon Seminar 5 -- Conference Hall A
(Sponsored by Hitachi High-Technologies Corporation)

Luncheon Seminar 6 -- Conference Hall B
(Sponsored by Agilent Technologies)

LS-1

LC/MS/MS tools for complex glycan and glycopeptide characterization in bio-therapeutics

Matthew M. Champion

Ph.D. Senior Scientist, Applied Biosystems Foster City, CA

The analysis of carbohydrates is of high importance in modern biochemistry. The extent to which carbohydrates govern the function of cellular processes is poorly understood. Glycoylation represents an ultimate post-translational modification, possessing tertiary structure, heterogeneity, complex structure-function determination and sub-stoichiometric labeling. Structural determination requires a breadth of knowledge involving sugar chemistry, sequencing, anomeric linkage understanding, and protein chemistry. Here we present a suite of LC/MS/MS tools and software for addressing many of the challenges associated with analytical characterization of glycosylation including those utilized in therapeutic Mab characterization with the 4800 TOF/TOF? and the QStar? Elite. Targeted identification and quantification of glycans and identification of glycopeptides is facilitated by utilizing the hybrid-scan modes of the 4000 QTrap? LC/MS/MS system.

LS-2

Brand new biomarkers for prostate cancer and diabetes

Bradley Walsh

CEO Minomic Pty Ltd

Proteomics has been recognised for some years now as being a useful way to discover new biomarkers for disease. Minomic was founded with the intention of deriving biomarkers from body fluids (such as serum, plasma, urine, tears, saliva and CSF) as well as providing pharmacoproteomics services to industry and research institutes.

Using the platform, known as MiNavigator^{TM}, we have developed biomarkers for both prostate cancer and type two diabetes. The prostate biomarker is useful for both diagnostic, imaging and therapeutic applications. The type two diabetes markers offer a new application in the diagnostic market by using proteins instead of blood sugar to detect diabetes. A pipeline of other products for cancer and metabolic diseases are also being researched. This talk will concentrate on how to discover new molecules and bring them to commercialization.

LS-3

New developments in ETD on LTQ Orbitrap XL and its applications

Justin Blethrow

Proteomics Marketing Specialist, Thermo Fisher Scientific

Electron transfer dissociation is a powerful fragmentation method that significantly improves the analysis of proteins and peptides, and their post-translational modifications (PTMs). We've now combined ETD with the high resolution and high accurate mass capabilities inherent in the LTQ Orbitrap hybrid mass spectrometer to create the most advanced proteomics platform on the market, especially suited for complex PTM analysis, top-down and middle-down analysis, intelligent sequencing of peptides, and relative protein quantitation via label-free differential analysis or stable isotope labelling such as with the new TMT (Tandem Mass Tagging) technology. New applications of ETD on the LTQ Orbitrap ETD hybrid FT mass spectrometer and its software for post acquisition data analysis will be introduced in this presentation.

LS-4

High definition mass spectrometry: A new way to visualise peptides, proteins and protein complexes in top-down/ bottom-up proteomics.

Mark A. McDowall

Waters Corporation

Ion Mobility Spectrometry (IMS) in dynamic combination with high resolution Time of Flight (TOF) Mass Spectrometry (MS) has emerged as an alternative strategy - enabling complex populations of ionised polypeptides, and their collision induced dissociation (CID) fragments, to be deconvoluted with high definition.

We have recently described a hybrid Quadrupole/IMS/TOF MS (Q/IMS/TOF MS) *"high definition mass spectrometry"* system that enables analysis of polypeptides based upon the combination of their ion mobility and m/z ratio [1,2]. Q/IMS/TOF MS analysis enables the collision cross-section of intact proteins to be calculated and the stoichiometry of protein complexes to be determined. Furthermore IMS enables CID fragment ions generated from intact proteins (or tryptic peptides) to be fractionated by charge state - greatly simplifying the resulting MS/MS spectra. This seminar has mainly these applications by using this instrument: SYNAPT ^{TM}HDMS^{TM}

LS-5

Electron capture dissociation in RF trap based ECD - TOF mass spectrometer

Takashi Baba, **Gary L. Glish**

University of North Carolina at Chapel Hill

Electron capture dissociation (ECD) tandem mass spectrometry (MS/MS) has become a powerful method to obtain peptide and protein sequence information as well as identification of post translational modification sites. Since the first linear RF ion trap based ECD was reported by T. Baba et al 2004, it shows excellent fast performance that is applicable to LC measurements. This ECD device is equipped in a linear ion trap-TOF mass spectrometer, "nano-Frontier ELD", released by Hitachi High Technologies. We will introduce our recent progresses on the ECD mass spectrometer as well as future applications on electron - ion reactions.

LS-6

HPLC-Chip combined with triple quadrupole MS for high sensitivity peptide quantitation

Ning Tang, Yanan Yang, Christine Miller, **Keith Waddell**

Agilent Technologies, Santa Clara, CA, USA

Multiple-reaction monitoring (MRM) on a triple quadrupole (QQQ) mass spectrometer provides superior sensitivity and selectivity for targeted peptides in a complex biological sample. MRM also offers high precision in quantitation and fast scan speed, which makes it an ideal technology for validating biomarkers in a high-throughput fashion. In this study, we confirm the methodology by spiking standard proteins into human plasma at various concentrations representing the wide dynamic range that would be seen in that biological fluid. Removal of the most abundant proteins in plasma was accomplished using the multiple affinity removal system for immunodepletion. The standard proteins were digested in silico using Spectrum Mill Peptide Selector to predict the peptides and their optimum MS/MS product ions. The spiked plasma samples were analyzed by robust and reproducible nanoflow LC/MS using the HPLC-Chip/MS interfaced to a high performance QQQ mass spectrometer. We will refer to other topics: "Analysis of Intact Antibodies using HPLC-Chip technology combined with True High Definition QTOF Mass Spectrometry" if we have time.

INDEX

N

O

P

R

MPSA2008 Secretariat

Prof. Kazuyasu Sakaguchi
Department of Chemistry, Faculty of Science, Hokkaido University
North 10, West 8, Kita-ku, Sapporo 060-0810 JAPAN
Phone: +81-11-706-2698 Fax: +81-11-706-4683

mpsa2008@sci.hokudai.ac.jp

Sapporo Campus Map

Scenic Campus